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Table of Contents

	Page
Spray Retention by Bermudagrass. B. E. Day and L. S. Jordan	351
The Effect of Environment on the Comparative Resistance of Petiole Parenchyma to Petroleum Oils. Wm. Harold Minshall	356
Cytochrome C Oxidase in the Tuber of Nutsedge (*Cyperus rotundus* L.). Rupert D. Palmer	364
Control of Madrone and Tanoak Stump Sprouting. Burgess L. Kay, Oliver A. Leonard, and James E. Street	369
Absorption and Distribution of EPTC—S^{35}. Shogo Yamaguchi	374
Effects of Surfactants on the Herbicidal Activity of Several Herbicides in Aqueous Spray Systems. L. L. Jansen, W. A. Gentner, and W. C. Shaw	381
Variation in Morphological Characteristics and Dalapon Susceptibility within the Species *Setaria lutescens* and *S. Faberii*. P. W. Santelmann and J. A. Meade	406
Soil-incorporated Pre-planting Herbicides for Witchweed Control. E. L. Robinson	411
Effect of Herbicides on Salmonberry and on Sitka Spruce and Western Hemlock Seedlings. James T. Krygier and Robert H. Ruth	416
Control of Field Bindweed and Other Perennial Weeds with Benzoic and Phenylacetic Acids. A. F. Wiese and H. E. Rea	423
Breaking Dormancy in Seeds of *Cuscuta approximata*. D. C. Tingey and Keith R. Allred	429
Effect of 3–Amino–1,2,4–triazole and L–Histidine on Rate of Elongation of Root Hairs of *Agrostis alba*. Wm. T. Jackson	437
The Decomposition of Amitrole in California Soils. B. E. Day, L. S. Jordan, and R. T. Hendrixson	443
Effects of Reduced Sunlight on Water Stargrass (*Heteranthera dubia*). Robert E. Wilkinson	457
Persistence of Soil-incorporated EPTC and Other Carbamates. L. L. Danielson, W. A. Gentner, and L. L. Jansen	463
Dissipation of Simazine from the Soil. O. C. Burnside, E. L. Schmidt, and R. Behrens	477
Studies in the Life History of *Artemisia absinthium*. G. W. Selleck and R. T. Coupland	485
A Carbon Dioxide Pressured Portable Field Sprayer. D. T. Lillie	491
Breaking Wild Oat Dormancy with Gases at High Pressure. Otto L. Hoffmann	493
Plant Population and Weed Growth Relations in Canning Peas. G. A. Marx and D. J. Hagedorn	494
Sustaining Members	497
News and Notes	498
Bibliography of Weed Investigations, October to December, 1960	499

Advertisers Index

Wiley & Sons	ii
Stauffer	iii
Chipman	iv
Spraying Systems	v
DuPont	vi

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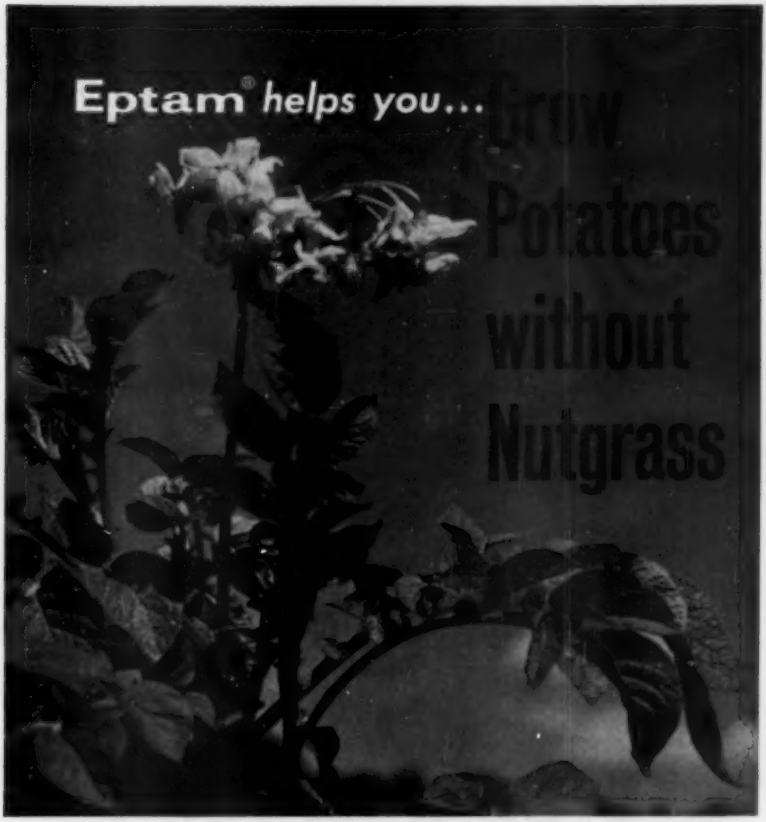

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Weeds

JOURNAL OF THE WEED SOCIETY OF AMERICA

VOLUME 9 JULY 1961 NUMBER 3

Spray Retention by Bermudagrass[1]

B. E. DAY and L. S. JORDAN[2]

IN field practice much of the application of herbicides to local infestations of bermudagrass (*Cynodon dactylon* L.) is done by hand on a spray-to-wet basis. With a fixed concentration of active ingredient in the spray mixture the dosage is determined by the volume used in wetting a particular stand of grass. The amount of herbicide deposited on the weed foliage may be affected by the addition of surfactants to the spray mixture (1). In orchards and vineyards where the tolerance of the crop to the herbicide is limited, variations in the stand may lead to local overdosage and injury to the trees and vines.

As an approach to better control of dosage, this paper presents information on the capacity of stands of bermudagrass to retain water and surfactant solutions and on the quantity and distribution of the liquids on the grass as affected by the growth habit and total amount of vegetation.

METHODS

Bermudagrass in an avocado orchard was mowed to one-half inch stubble and allowed to regrow to an average height of four to five inches. One-foot-square plots chosen at random were clipped back to the stubble and the fresh weight of the grass was determined. Retention of water by the grass was determined by weighing individual shoots suspended from a hook on a balance and then dipping the shoots in distilled water or water containing varying amounts of the surfactant sodium dioctylsulfosuccinate[3] and weighing again after the last drop of runoff had fallen. Total water retention per acre of grass was then calculated. Ten randomly selected shoots were used in each determination.

The grass from ten plots was clipped to one-half-inch height and weighed. Randomly chosen shoots were weighed, dipped in 0.01 percent surfactant solution, and weighed again after runoff had

[1]Received for publication August 19, 1960. Paper No. 1282, University of California Citrus Experiment Station, Riverside, California.

[2]Associate Plant Physiologist and Assistant Plant Physiologist, respectively, Department of Horticulture, University of California Citrus Experiment Station, Riverside, California.

[3]Vatsol OT. This was the surfactant used throughout this investigation.

stopped. Dead tissues were removed from the shoots which were then wiped dry with absorbent tissue, and the procedure of weighing, wetting, and reweighing was repeated. Shoots from which dead tissues had been removed could be wiped dry and restored to their original weight. Next the leaf blades were removed and the solution retention of the stems with enclosing leaf sheaths was determined. In this way the amount of solution held by the whole shoot and the proportion held by the leaf blades, stems, or dead tissues was individually estimated.

The effect of variation in growth habit on water retention by bermudagrass was also measured. The stand and growth habit of bermudagrass is known to vary with environmental conditions (2). Under arid or low-nitrogen conditions the runners are thickened and woody and bear fewer and smaller leaves having shortened leaf sheaths. Under fertile, moist conditions the stems are small and have many large leaves. There are always partially dead, dry leaves clinging to the green shoots. The effect of some of these variations on the amount and distribution on the tissues of retained water was measured in four selected stands of grass as follows: (A) coarse, stubby growth, of low vigor, 3 inches tall, dry conditions; (B) grass of medium vigor, 5 inches tall, slightly moist conditions; (C) coarse runners 10 to 15 inches long, leaves few and small, dry conditions;

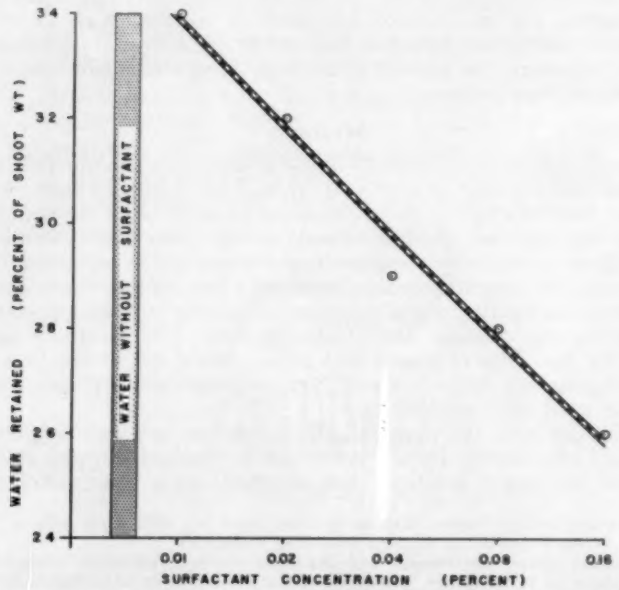

Figure 1. Retention by bermudagrass runners of distilled water and water containing a series of concentrations of sodium dioctylsulfosuccinate as surfactant.

and (D) vigorous, dark green, young growth, 7 inches tall, which had grown rapidly following mowing in a moist, fertile location.

RESULTS

The mean fresh weight of grass from 10 plots was 7537 ± 494[4] lb/A. Average retention of distilled water was 35.9 percent of fresh shoot weight and of water containing 0.01 percent surfactant was 41.1 percent of fresh weight. Calculated wetting volumes are 325 gal/A for water and 371 gal/A for water with surfactant. The difference of 46 gal/A is not significant although the manner of water retention was visibly different. With surfactant added, the water spread over the plant surfaces as a wetting film, but water alone was held largely as discrete droplets, usually in the leaf axils. Thus when surfactant was present water retention was more uniform over a given shoot and likewise less variable from shoot to shoot. The standard deviation for retention per shoot was 16.5 percent of shoot weight for water alone and 7.5 percent for water plus surfactant. The difference between these standard deviations and thus the difference in uniformity of wetting is significant at a confidence level between 97.5 and 99 percent.

The effect of a range of concentrations of Vatsol OT on water retention is given in Figure 1. Here average retention of both water and 0.01 percent surfactant solution was 34 percent of fresh shoot

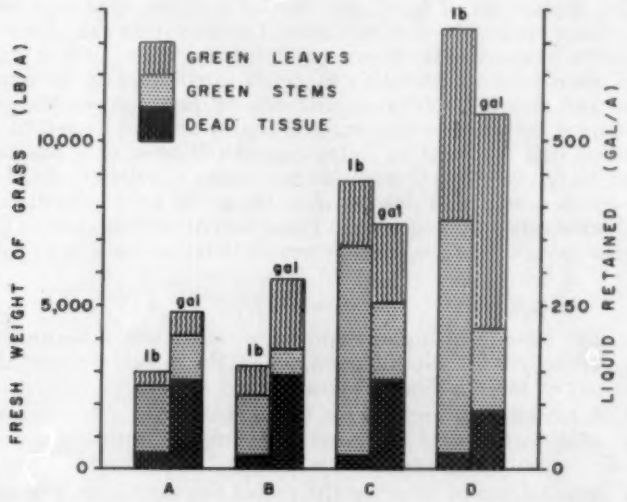

Figure 2. Fresh weight and retention of 0.01 percent sodium dioctylsulfosuccinate solution for four stands of bermudagrass subdivided into three classes of plant materials. Standard errors for total spray volume are 5.8, 5.6, 6.5, and 7.8 percent of spray volumes, respectively, for stands A, B, C, and D.

[4]Standard error of the mean.

weight, corresponding to 307 gal/A. Higher concentrations of surfactant resulted in greater runoff; 0.16 percent surfactant reduced retention to 26 percent of fresh shoot weight or 236 gal/A.

Retention of 0.01 percent surfactant solution by the four stands of grass is shown in Figure 2. The volume of liquid held increased as total plant material increased but not in direct proportion. This was evidently due to the differences in the relative amounts of the different tissues present, particularly of the dead leaves, which retained about ten times more water per unit weight than the stems and two to four times as much as the green leaves. Fresh weight of plant material varied as much as 4.6-fold (from 2930 lb/A to 13,400 lb/A) while water retention varied as much as 2.2-fold. Since the amount of dead material per acre was relatively constant and held on the average about 125 gal/A, the amount of liquid retained by living tissues varied to about fourfold from 111 gal/A to 449 gal/A.

DISCUSSION

It is not proposed that the values for liquid retention obtained from dipping tests are identical with volumes required to wet vegetation by spraying in the field. The efficiency factors and spray wastage inherent in field spraying, as pointed out by Fraser (3), plus the variations in subjective judgment by the applicator as to the degree of wetting must be superimposed on the data reported here.

The limitations of spraying-to-wet as a means of dosage control are clearly evident in that surfactant concentration can affect spray retention by one-fourth or more, and differences in stand of grass by more than twofold. Probably of equal importance is the effect of stand and growth habit on distribution of the liquid on the plants. In stand A the solution was retained largely on dead tissues. In stand B about half was held by living tissues and most of it was on the green leaves. In stand C retention was about equally divided among the leaves, stems, and dead tissues. On stand D the retention was predominantly by green leaves. These variations would be expected to greatly influence the effectiveness of foliar systemic herbicides.

SUMMARY

1. The amount of liquid required to wet stands of bermudagrass was determined by dipping samples of the grass in water and in solutions of sodium dioctylsulfosuccinate.

2. Retention was greatest for distilled water and low concentrations of surfactant and decreased by about one fourth as surfactant concentration was increased from 0.01 to 0.16 percent.

3. Distribution of water on the shoots was significantly more uniform when surfactant was present.

4. In four populations of bermudagrass studied, retention of 0.01 percent surfactant solution ranged from 247 gal/A to 536 gal/A with wide variations in the proportion of the liquid held by the green leaves, green stems, and dead tissues.

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The Effect of Environment on the Comparative Resistance of Petiole Parenchyma to Petroleum Oils[1]

WM. HAROLD MINSHALL

INTRODUCTION

RECENT reviews [Crafts (1), Currier (2), Woodford (7)] dealing with the mode of action of phytotoxic chemicals claim the acute toxicity of petroleum oils to plant tissues is due to the destruction of the plasma membrane of the cell. Although considerable evidence supports such a claim, much of it rests on results from the effect of vapors on greenhouse grown plants rather than on the effect of liquid oils on field grown plants. Of the numerous workers who have noted a selective action of oils on different plant species, most of them determined the selectivity by visual observation of injury. Currier and Peoples (3), Dallyn (4), and Van Overbeck and Blondeau (6) linked the selective action by experimentation to a differential effect on a cellular level but only the work of Currier and Peoples included direct determinations of cellular vitality. It seemed desirable, therefore, to obtain further information on the relative susceptibility to petroleum oils of field-grown plants.

Details of the methods were developed with plants grown in the greenhouse in early spring before the field-grown material was available. When the high temperatures common to greenhouses in the summer months started to affect growth, the plants were transferred outside to a coldframe. Plants from this coldframe exhibited a higher resistance to oils than did plants from the field or the greenhouse, which suggested that certain factors of the environment might alter the relative resistance of plants. To check this suggestion, tests were made on plants grown under different light panels in a controlled environment room.

MATERIALS AND METHODS

The oil used in these studies consisted of different proportions of the aromatic hydrocarbon tetrahydronaphthalene (tetralin) in a non-phytotoxic paraffinic petroleum oil.[2] Of 31 petroleum hydrocarbons tested by Havis (5) tetralin was one of the most toxic to plants and a 15% concentration of this hydrocarbon in a nontoxic oil gave selective toxicity with carrots. As it is generally agreed that aromatic content is one of the important characteristics determining selective toxicity of petroleum oils, the mixture of tetralin in the paraffinic oil was considered to be representative of the petroleum naphthas used for controlling weeds in umbelliferous crop plants.

A single source of practical grade tetralin (Eastman P550) stored in a dark colored bottle was used throughout the study. Tetralin has

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[2]Bayol D.

a boiling point of 405° F. The characteristics of the paraffinic oil were as follows: A.P.I. gravity—48.1°, viscosity—34 sec. at 100° F, boiling range—394–512° F, aromatic content—0. In preliminary tests mixtures of 0, 5, 10, 20, and 50% of tetralin required $>$ 360, 120, 75, 50, and $<$ 10 minutes, respectively, to kill 50% of the parenchyma cells of parsnip petioles.

The plant material used included two species resistant to herbicidal oils, parsnip (*Pastinaca sativa* L., var. Hollow Crown) and carrot (*Daucus carota* L., var. *sativa* DC. var. Nantes Half Long Coreless): and two species susceptible to oils, beet (*Beta vulgaris* L., var. Detroit Red) and field mustard (*Brassica kaber* (DC.) L. C. Wheeler, var. *pinnatifida* (Stokes) L. C. Wheeler). In order to vary the environment these plants were grown in the field, greenhouse, coldframe, and under different lighting panels in a controlled environment room.

All field-grown plants were in the open and well exposed to the sun. Weekly plantings of parsnip, carrot, and beet in plots provided plants of the desired age for tests during the summer months. For mustard, volunteer plants occurring in the plot area and nearby flower beds were used. The tests on mustard were made in May and June, while this species was in a vegetative condition.

In the greenhouse, in the coldframe, and in the controlled environment rooms, weekly plantings were made in flats 11″ long, 7″ wide and 3″ deep. The seedlings were thinned to 12 to 15 plants of approximately the same stage of development in each flat. Plants grown in the greenhouse were subject to regular greenhouse practice without supplementary light. The coldframe had a protected southern exposure resulting in high temperatures.

In the controlled environment room the plants were grown under a 16 hour light period, 8 hour dark period and steady state 70° F and 45% R.H. Three different conditions of lighting were set up in this room called in this paper (1) X light panel, (2) Y light panel, and (3) H light panel. The X panel consisted of T8 slimline standard cool white fluorescent lamps at 2 inch centres and 60 watt neck reflector incandescent lamps at 24 inch centres. The Y panel also consisted of T8 slimline standard cool white fluorescent lamps at 2 inch centres, but the 60 watt incandescent lamps were at 14 inch centres, and in addition, for 4 hours in the middle of the light period supplementary 100 watt neck reflector incandescent lamps at 7 inch centres were turned on. When not lighted these supplementary lamps reduced the intensity of the regular panel. The H panel was similar to the X panel but with supplementary 300 watt reflector spot incandescent lamps at 6 inch centres and Chance No. 20 heat absorbing filters placed 12″ above the level of the soil. This filter absorbs 95% of the infrared energy and transmits 85% of the visible light. The conditions of temperature and light intensity under the three panels are given in Table 1.

All values given in this paper are LD_{50} time values and represent the length of exposure to the tetralin dilutions required to kill 50% of the ground parenchyma cells of petiole tissue. Cells of the outer

Table 1. Temperatures and light conditions under the three light panels in a controlled environment room operated at steady state 70° F and 45% R.H. Descriptions of light panels are given in the text.

Light panel	Light condition	Light intensity		Temperature fahrenheit by shaded thermocouples		
		Ft-c	G cal / cm² x min	Air under 3rd leaf	On soil surface	Soil at bottom of flat
X	Light	1400	0.15	77	77	76
	Dark	—	—	69	66	64
Y	Light with supplementary 100 Watt lamps	2500	1.30	91	98	90
	Light, regular	1000	0.15	74	73	70
	Dark	—	—	67	65	62
H	Light	5000	0.42	85	78	72
	Dark	—	—	70	67	63

cortex and of the vascular sheath have longer survival times than do the ground parenchyma cells, but as it was more difficult to secure sections containing an adequate supply of cortical or vascular sheath cells, observations were limited to the plates of parenchyma cells between the vascular strands in the central portion of the petiole. Because parsnip and carrot have some vascular bundles scattered throughout the parenchyma, the plates of parenchyma in these two species contained fewer rows of cells than did those of mustard or beet. In addition many of the parenchyma cells of parsnip and carrot were somewhat narrower and longer than were those of mustard and beet.

With plants from the field, greenhouse, or coldframe all determinations were made on petioles of either the third or the fourth leaf. For the most part these leaves were taken soon after reaching full size. The LD_{50} of parsnip parenchyma from plants grown in the controlled environment room increased with the age of the leaf. A similar increase did not occur in parsnip or beet from the field, greenhouse, or coldframe nor in beet plants from the controlled environment room. The LD_{50} did not vary appreciably while the parsnip petiole was elongating but it increased with age after the petiole had attained its full length. Although the effect of age appeared to be confined to parsnip all tests on controlled environment room grown beet and parsnip were made on the third leaf. The LD_{50} of the third leaf of the plants in each flat was determined as the leaf reached full size and again 5 or 6 days later and the two results were averaged. The size of a succeeding leaf (with parsnip the fourth leaf and with beet the fifth leaf) was used as a guide in determining the sampling date for the third leaf.

As soon as possible after the plants were removed to the laboratory, thin longitudinal sections of petiole were cut free hand with a sharp razor and the sections placed in distilled water. The sections from the outer part of the petiole containing mostly cortical cells were discarded. When 30–35 sections had been cut, they were removed from the water, blotted on filter paper, and placed in a 4.5

cm preparation dish containing approximately 10 ml of the oil. At intervals of 10–20 minutes three sections were removed from the oil, blotted on filter paper and placed in 10 ml of the vital stain neutral red at a concentration of 20 ppm in 0.04 M $CaCl_2$ and made slightly orange in color with NaOH. After 60 minutes in the stain the sections were removed and mounted in a drop of $CaCl_2$ plasmolyte on a microscope slide. The strength of the plasmolyte was varied from 0.1 to 0.3 M as required to produce only a moderate degree of plasmolysis. Parsnip and carrot cells required a higher concentration of plasmolyte than did beet or mustard cells. After standing in the plasmolyte for 30 minutes the sections were observed under the microscope for neutral red uptake and for plasmolysis. Glass loops or needles were used to handle the sections at all times.

Parenchyma cells of parsnip and carrot did not take up neutral red as readily as did those of mustard or beet but they usually absorbed sufficient of the stain to facilitate observation. Beet cells contained a natural red stain in their vacuoles but to maintain a uniform procedure, the beet cells also were exposed to the vital stain. Once the sections were exposed to the oil the number of dead parenchyma cells increased gradually and continuously with time and for this reason it was necessary to maintain uniform time intervals in each stage of the procedure. Control sections were carried in water to make certain death was caused by the oil treatment and not by other stages of the procedure.

Table 2. The time required to kill 50% (LD_{50}) of the ground parenchyma cells of leaf petioles from plants grown in the field when exposed to 5 and 10% tetralin in a paraffinic oil.

Plant	Number of tests	LD_{50} of petiole parenchyma, min	
		5% tetralin	10% tetralin
Mustard	15	50	35
Beet	6	60	40
Carrot	10	>80	65
Parsnip	6	>90	85

Although the tetralin was stored in a dark colored bottle in low light intensity, it was noted towards the end of its two years of use that a longer exposure time was required to kill the parenchyma cells. This unexpected deterioration of the tetralin means that only the results from experiments conducted close together in time can be used for direct comparisons. The deterioration was not noticed until more than three-quarters of the contents of the bottle had been used and it was accompanied by the appearance of a brownish coloration.

RESULTS

Parenchyma cells from the petioles of field grown plants of parsnip and carrot had higher LD_{50} values to tetralin solutions than did similar cells of mustard or beet (Table 2). Likewise petiole parenchyma cells of parsnip plants grown in the coldframe or in a con-

trolled environment room had higher LD_{50} values than did similar cells of beet (Table 3). Except for the 25 tests with plants grown in the greenhouse, parsnip and carrot consistently had higher LD_{50} values than did beet and mustard in the 300 separate tests made over a two-year period. These results indicate that individual parenchyma cells of the umbelliferous plants, parsnip and carrot, possess a greater resistance to petroleum oils than do the cells of mustard and beet.

Table 3. The effect of different environments on the resistance of parenchyma cells of parsnip and beet petioles to 10% tetralin in a paraffinic oil.

Environment	Tests made	LD_{50} of petiole parenchyma, min	
		Beet	Parsnip
Greenhouse	Apr.–May	40	40
Field	July–Sept.	40	85
Coldframe	July–Sept.	65	90
Controlled environment room, X panel	Nov.–Dec.	40	210
Control environment room, Y panel	Nov.–Dec.	80	200

That environment might affect the resistance of parenchyma cells to petroleum oils was first suspected when it was noted that beets grown in the coldframe had an LD_{50} of 65 minutes compared to 40 minutes for plants grown in the field (Table 3). Under appropriate conditions, however, an even greater difference than this was evident, for while the average of the 11 tests made with beets grown in the coldframe was 65 minutes, one flat had plants with an LD_{50} of 100 minutes. As plants in both the coldframe and the field were subject to full sunlight, the light intensity should have been approximately the same but because of its protected location temperatures were higher in the coldframe than in the field. When the possible effect of temperature was investigated in the controlled environment room, the higher temperature under the Y panel resulted in an increased resistance of beet cells (Table 3). However, as the Y panel subjected the plants to higher light intensities as well as higher temperatures, a further test of these two factors was made by means of the H panel where the light intensity was higher than both X and Y but the temperatures were midway between those under X and Y (Table 1). The results for plants grown under the H panel in February and March are not included in Table 3 for it was obvious that the deterioration of the tetralin was prolonging the values. At this time, the LD_{50} for beet parenchyma from plants grown under panels X, H and Y was 45, 65 and 85 minutes, respectively. That is, the LD_{50} of the cells was higher as the temperature was higher. Temperature, therefore, and not light intensity per se, appeared to be the factor responsible for the increased resistance of beet cells.

Parenchyma cells of parsnip plants grown in the controlled environment room had much higher LD_{50} values than did cells of plants grown outside in the field or coldframe (Table 3), suggesting that some factor in the environment affected the resistance of parsnip cells. Temperature, the factor responsible for increased resistance in beet, apparently had little effect on parsnip cells as the resistance of

plants grown in the coldframe and in the field was approximately the same and that of plants grown under X and Y panels in the controlled environment room was approximately the same. Apparently some as yet unknown factor in the controlled environment room increased the relative resistance of parsnip cells.

Because of the differential effect of factors of the environment on the resistance of cells of different plant species it would appear that the degree of selectivity can be varied according to the environment. In the present studies the ratio of the LD_{50} values of parsnip to those of beet was 1, 1.5, 2, and 5 for plants grown in the greenhouse, coldframe, field, and under X panel of the controlled environment room, respectively.

Cell size: Some of the beet plants grown in the coldframe which exhibited the high LD_{50} values had dark red colored leaf blades and short petioles. When individual parenchyma cells from these leaves were measured they averaged 15 to 20 μ shorter than similar cells from field grown plants. This difference, however, represented less than 10% of the total length of the cells. Furthermore cells from different plants grown in the coldframe varied from 96 to 340 μ in length while those from plants grown in the field varied from 88 to 280 μ in length. Cells from petioles of plants grown under the Y panel in controlled environment rooms averaged 185 μ in length compared to 210 μ for those grown under X panel, but the cells from Y panel varied from 105 to 315 μ in length while those from X panel varied from 105 to 395 μ. There did not appear to be any consistent relationship between cell size and the increased resistance of beet parenchyma cells to the oils.

Deterioration of tetralin. As mentioned previously, increased LD_{50} values were encountered in the later stages of this study, caused presumably by some deterioration of the tetralin. It is possible, of course, that this deterioration had started during the 9 months covered by the results given in Tables 2 and 3, but since the LD_{50} of beet was identical in the greenhouse, the field and under X panel of the controlled environment room, it is believed that the effects from this deterioration form a very small part of the differences reported between species and treatments.

The effect of the deterioration of the tetralin was less noticeable on beet than on parsnip. From November to April under the X panel in the controlled environment room the LD_{50} of beet to 10% tetralin changed from 40 to 45 minutes, an increase of 12%, while that of parsnip changed from 210 to 315 minutes an increase of 50%.

DISCUSSION

The present study, wherein the time required to kill parenchyma cells with petroleum oils was determined by plasmolysis tests, supports the general theory that the toxic action of such oils is due to the destruction of the cell membranes. Furthermore, since individual parenchyma cells from petioles of field grown parsnip or carrot had longer LD_{50} times than did cells of mustard or beet, the toler-

ance to oils by parsnip and carrot under field conditions was evident on a cellular level. Selective action of petroleum oils between resistant and susceptible plants is apparently the result of a relative protection of the plasma membrane for different periods of time. Unfortunately the results from the present study do not delimit the part of the cell responsible for the resistance, because the protective action encountered could have been provided by the cell wall equally as well as by the ectoplast or the plasma membrane itself. Perhaps, since the factor or factors of the environment found to change the resistance of beet differed from those required to change the resistance of parsnip, the total resistance of a cell may be composed of more than one component with contributions from different cell parts.

Although, as stated previously, it is not considered that the deterioration of the tetralin substantially affected the results given in Tables 2 and 3, it is possible that this deterioration may account for some of the increase in LD_{50} values of parsnip plants grown in the controlled environment room in December over plants grown in the greenhouse in April. This is suggested by the fact that the LD_{50} of parsnip continued to increase considerably in the late stages of the study when deterioration was known to be occurring. Under these conditions, however, it was obvious that the resistance of parsnip increased to a much greater extent than did that of beet. Therefore, even if one assumes the view that a large part of the difference in the LD_{50} of parsnip was caused by the deterioration of tetralin, the results still show different degrees of resistance between the two species, parsnip and beet.

SUMMARY

By neutral red uptake and plasmolysis in calcium chloride the length of time required for 10% tetrahydronaphthalene in a paraffinic oil to kill 50% (LD_{50}) of the ground parenchyma cells from petioles of field grown mustard [*Brassica kaber* (DC.) L. C. Wheeler, var. *pinnatifida* (Stokes) L. C. Wheeler], beet [*Beta vulgaris* L.], carrot [*Dancus carota* L. var. *sativa* DC.], and parsnip [Pastinaca sativa L.] was 35, 40, 65, and 85 minutes respectively. Thus the greater tolerance of the umbelliferous plants, carrot and parsnip, to petroleum oils was evident on a cellular level and appeared to arise through an increased resistance or protection of the cellular membranes.

Increased temperature increased the resistance of parenchyma cells of beet to 10% tetrahydronaphthalene in paraffinic oil but had little effect on the resistance of cells from parsnip. Likewise, when plants grown in a controlled environment room were compared to those grown in the field, the LD_{50} of parsnip cells was markedly increased while that of beet remained approximately the same. Because of the differential effect of environmental factors on the resistance of these species, the ratio of LD_{50} values of parsnip to those of beet was 1, 1.5, 2 and 5 for plants grown in the greenhouse,

cold frame, field and controlled environment room, respectively. As factors of the environment differed in the extent to which they changed the resistance of parenchyma cells of parsnip and of beet to petroleum oils, it is suggested the total resistance of a plant cell may involve more than one component with contributions from different parts of the cell.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the assistance of Mrs. Evelyn Hunt whose preparation and handling of sections and persistent pursuit of plasmolysed protoplasts made possible the determination of the LD_{50} values. R. Belton and J. Broer assisted in growing the plants.

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Cytochrome C Oxidase in the Tuber of Nutsedge (*Cyperus rotundus* L.)[1]

RUPERT D. PALMER[2]

THE general acceptance of cytochrome oxidase as the terminal link between the various dehydrogenase systems and oxygen in plants was stated in the reviews by Smith and Chance (6), James (4), and Hartree (2).

Recent distribution surveys of various cytochromes and cytochrome oxidase have been conducted by Webster (8). He extracted an enzyme which oxidized cytochrome c from the tissues of 54 different species which represented 23 families of dicotyledonous plants. Webster (9) found this cytochrome oxidase activity to be strongly inhibited by carbon monoxide in the dark and the inhibition was reversed by light. Cytochrome oxidase activity was found in most cases to be associated with a "particulate" fraction of the cell material (2, 4, 8).

MATERIALS AND METHODS

The tubers used in these studies were grown as previously described (5). The tubers were placed on filter paper in Petri plates. Five mls of full strength Hoagland's general culture solution at pH 7.1 were added to each plate. The plates were placed in a dark incubator set at 28° C to germinate.

I. Preparation of "particles". The tubers were homogenized and the homogenate was centrifuged as previously reported (5). The buffer was used at pH 7.4 and contained 0.1 M potassium phosphate, 0.65 M sucrose, 1.3×10^{-3}M EDTA (ethylenediaminetetraacetic acid) and 5.3×10^{-4} M $AlCl_3$. The "particles" were used immediately after being dispersed in the buffer.

II. Preparation of cytochrome c. The cytochrome c was reduced with sodium hydrosulfite (1) and the concentration of reduced cytochrome c was determined colormetrically (3).

III. Estimation of enzyme activity. Oxygen uptake was determined by conventional Warburg methods (7). The gas mixtures were prepared and the necessary gas atmosphere within the experimental vessels was obtained by the replacement technique (7).

For dark studies, the vessels were placed in beakers of a concentrated aqueous solution of gentian violet at 25° C. For light effects on the reaction, the vessels were set over a 22-watt fluorescent tube[3] mounted in the Warburg water bath.

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[3]The author wishes to acknowledge the help of Mr. Fred Shuman, Department of Agricultural Engineering, Mississippi State University, for his assistance in adapting this light for the Warburg apparatus.

IV. Cytochrome c oxidase on slices 40 μ thick and associated with particles. A 0.5 ml aliquot of cytochrome c was placed in the side arm and tipped into the vessel after equilibration. The vessels contained either 30–40 slices in 1.5 ml of buffer of the following composition: 0.1 M potassium phosphate, 0.5 M sucrose, 4×10^{-4}M $AlCl_3$, and the same concentration of EDTA as stated above for "particles", or 1.5 ml of "particles". The final concentration of cytochrome c was 1.5×10^{-6} M. When cytochrome c was omitted 0.5 ml of 0.1 M potassium phosphate buffer was tipped into the vessel.

V. Preparation of dry tissue and chemical methods. Dry weight of tissue slices and determination of nitrogen in the "particle" suspension were carried out as previously described (5).

RESULTS

I. Carbon monoxide inhibition of oxygen uptake by slices. A preliminary study to determine if carbon monoxide would inhibit the oxygen consumption of slices of nutsedge tubers which had germinated for 2 days was run under fluorescent laboratory light. The oxygen consumed for an atmosphere of 95% CO and 5% O_2 was 105 μl per gm dry wt per hr, while the oxygen consumed by an identical tissue sample from an atmosphere of 95% N_2 and 5% O_2, which served as a control, was 182 μl. Carbon monoxide caused a significant (43 percent) inhibition of oxygen uptake in this test.

II. Carbon monoxide inhibition of oxygen uptake by tissue slices during alternate dark and light periods. The rate of oxygen uptake by tissue in an atmosphere of 95% CO and 5% O_2 in the dark for 30 minutes was significantly inhibited by 41 percent (Table 1). The

Table 1. Carbon monoxide inhibition of oxygen consumption by slices, 40 μ thick, from nutsedge tubers during alternate dark and light periods and its reversal by light. Tubers had germinated for 2 days.

Minutes in dark or light	O_2 uptake/gm dry wt, μl				Percent inhibition	
	Experimental atmosphere 95% CO—5% O_2		Control atmosphere 95% N_2—5% O_2			
	Dark	Light	Dark	Light	Dark	Light
30	31	—	53	—	41	—
70	—	143	—	147	—	3
40	174	—	212	—	18	—

same tissue in the same gas mixture in the light for 70 minutes showed less inhibition (3 percent). When the vessels containing the same tissue in the same atmosphere were placed in the dark again for 40 minutes, the inhibition increased from 3 to 18 percent. This demonstrates the photo-reversibility of the respiratory inhibition produced by carbon monoxide.

III. The effect of reduced cytochrome c upon oxygen uptake by tissue slices. When the cytochrome c was tipped into vessels containing the slices, oxygen uptake was 245 μl per gm dry wt per hr; whereas,

the control consumed only 186 µl on the same basis. Cytochrome c gave a 24 percent stimulation of oxygen uptake.

IV. Effect of carbon monoxide upon oxygen uptake of tuber slices in the presence of cytochrome c during alternate dark and light periods. This study was designed to determine the effects of alternate dark and light periods on the same tissue in an atmosphere of carbon monoxide. The results of a typical experiment are plotted in Figure 1 A, B. It may be seen from Figure 1 A and B that initially there was a stimulation of oxygen uptake by tissue in vessels with the above treatment in the light, but when they were placed in the dark a striking inhibition of oxygen uptake occurred for this treatment; whereas, the tissue under nitrogen and oxygen exhibited a gradual increase in oxygen uptake. Upon returning the vessels with carbon monoxide and oxygen to the light there was a rapid reversal of the inhibition shown in the dark. This reversal is shown by the difference in the slope of the curve for carbon monoxide (Figure 1 A)

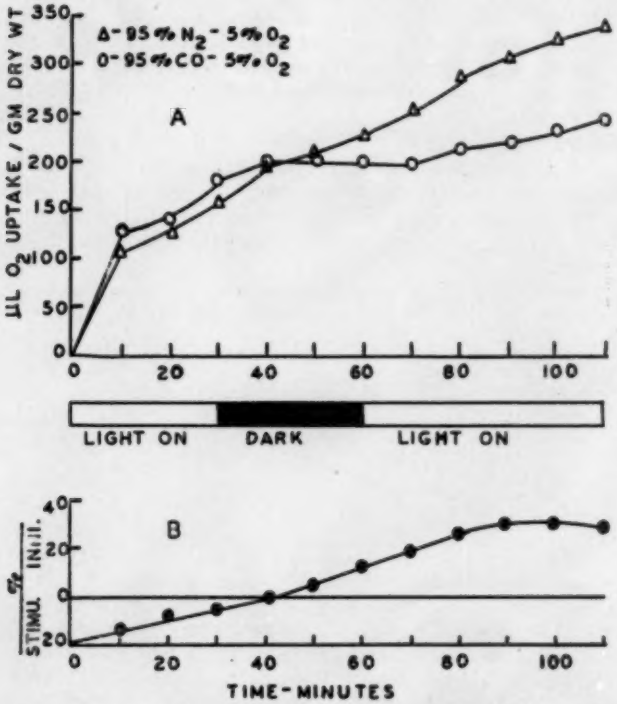

Figure 1. A—Effect of carbon monoxide on oxygen uptake by slices of nutsedge tubers during alternate dark and light periods. B—The percent stimulation or inhibition the carbon monoxide induced as compared to the nitrogen treatment. The tubers had germinated for 3 days.

and by the inhibition curve (Figure 1 B). This shows the photoreversibility of oxygen uptake which was stimulated by cytochrome c. There appears to be an incubation period for darkness to bring about inhibition and for light to initiate stimulation of oxygen uptake because 10 minutes was needed in each case as shown by the carbon monoxide and oxygen curve in Figure 1 A. Inhibition and stimulation of oxygen uptake by the tissue was not instantaneous in this case.

V. Activity of cytochrome c oxidase during germination. Because succinic dehydrogenase increased in activity during germination of the tuber (5), the activity of cytochrome c oxidase during germination was determined. This might show the activity relationship of the enzymes.

There was a gradual increase in activity of cytochrome c oxidase during germination. The oxygen uptake of "particles" from the dormant tuber was 25 μl per mg N per hour; whereas, at 5 days and 8 days oxygen uptake was 27 and 38 μl, respectively.

DISCUSSION

The dormant and germinated nutsedge tuber was shown to have an active cytochrome c oxidase. Previous studies (5) have shown that the nutsedge tuber also contained an active ascorbic acid oxidase and polyphenoloxidase. It has been shown (5) that polyphenoloxidase was very active in the dormant tuber and decreased during germination. From these studies, however, no conclusion can be drawn as to which of these enzymes mediates the terminal electron transfer in the nutsedge tuber.

SUMMARY

Cytochrome c oxidase was shown to operate in dormant and germinated nutsedge tubers. A CO/O_2 ratio of 19 showed inhibition of oxygen uptake by tissue slices of 41 percent, as compared to values obtained in a control atmosphere of nitrogen and oxygen (N_2/O_2 = 19). This inhibition was photo-reversible. The addition of cytochrome c to tuber slices resulted in a stimulation of 24 percent of oxygen consumption, which was demonstrated to be photo-reversibly inhibited by carbon monoxide.

The activity of cytochrome c oxidase increased during germination of the tuber.

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Control of Madrone and Tanoak Stump Sprouting[1]

BURGESS L. KAY, OLIVER A. LEONARD, and JAMES E. STREET[2]

MADRONE *(Arbutus menziesii Purch.)* and tanoak *(Lithocarpus densiflora* (H & A) Rehd.) are often considered undesirable along the Pacific Coast. These weed trees are removed to increase forage production; to remove competitors of more valuable timber trees, especially Douglas fir *(Pseudotsuga menziesii* (Mirb) Franco); or to obtain firewood or charcoal. Tanoak is occasionally cut for tanbark harvest. Control of stump sprouting following cutting would be desirable.

Both madrone and tanoak sprout vigorously after being cut. Sprout growth is so rapid that cutting these species provides only temporary release for other crop trees (4, 5, 6). Sprouts of both species may reach twelve feet in three growing seasons. Madrone or tanoak sprouts are rarely browsed heavily enough to control regrowth. Likewise they are difficult to control by repeated burning.

Killing stumps with herbicides is not new. Sodium arsenite and other chemicals were used before discovery of the phenoxy herbicides. Stump spraying with the esters of the latter herbicides in oil is widely used to prevent or reduce sprouting of stumps. Their effectiveness has been known since at least 1948 (1). The effectiveness of concentrates of the mixed alkanolamine salts of 2,4–D (2,4–dichlorophenoxyacetic acid) to prevent or reduce sprouting represents the basis of recommendations by Leonard and Harvey (3). The present study is a comparison of the effectiveness of the ester and amine treatments in preventing or reducing sprouting of madrone and tanoak.

METHODS AND MATERIALS

Madrone and tanoak grow in a region of warm to hot dry summers and mild wet winters with only occasional snow. Stumps were treated in each of the four seasons. The field work was at Garberville, California, 175 miles north of San Francisco and near the coast. Annual rainfall averages 56 inches there, with almost no precipitation between May 15 and October 1.

Treatments were on April 19, 1956, July 12, 1956, October 10, 1956 and February 6, 1957. Forty trees of each species were cut on each of these dates. Stems were cut at a twelve-inch stump height. If a group of stems appeared to be joined underground (probably sprouts from an earlier stem) they were considered as one tree. The number of stems per tree varied from 1 to 5 in madrone and 1 to 7 in tanoak, respectively averaging 1.6 and 1.8. Stem diameter twelve

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inches above the ground varied from 1 to 24 inches for madrone and 1 to 20 inches for tanoak.

Herbicides were applied, not more than one hour after tree cutting, to ten trees of each species at each date with the following treatments:

1) Mixed alkanolamine salts of 2,4–D (4 lb/gal ae), hereafter referred to as 2,4–D amine, daubed on the top of the stump with a paint brush. With stumps six inches or less in diameter, the entire top surface was covered with 2,4–D amine by daubing the brush up and down. More material is applied by daubing than by just painting. Larger stumps were treated in a 3- to 4-inch peripheral band on the top of the stump. The amount of 2,4–D applied averaged 74 cc per square foot of stump top, roughly equal to treating 260 six-inch stems with 1 gallon of 2,4–D amine.

2) Entire stump sprayed wet to the ground surface with a 16 lb aehg solution of 2,4–D butoxy ethanol ester, hereafter referred to as 2,4–D ester, in diesel oil.

3) Entire stump sprayed wet to ground with a total of 16 lb aehg of a solution containing equal parts of 2,4–D ester and the butoxy ethanol ester of 2,4,5–T (2,4,5–trichlorophenoxyacetic acid), hereafter referred to as 2,4,5–T ester, in diesel oil. The amount of spray material used in treatments 2 and 3 averaged 1 gallon of spray material for each 5.63 square feet of stump top. This is roughly equal to treating 30 stumps six inches in diameter with one gallon of spray solution.

4) Ten trees of each species were cut at each of the four dates and left untreated as a check.

RESULTS AND DISCUSSION

All stumps were observed for 3 successive years after treatment. The number, height, and condition of sprouts were recorded in June, 1957, August, 1958, and May, 1959.

New sprouts continued to appear on untreated stumps of both species following all cutting dates for two growing seasons. There was no increase in the total number of sprouts between the second and third growing season. Untreated tanoak stumps consistently produced half again as many sprouts as did madrone at all dates of cutting (Figures 1 and 2). Both species produced the greatest number of sprouts when cut in April, and the least when cut in February or July.

Average sprout height continued to increase throughout the three years measured. The differences in sprout length between species varied with the season at which the trees were cut. Average sprout length of check trees cut in February was more than twice as great for madrone as for tanoak, but tanoak had a greater mean sprout length than madrone with all other cutting dates (Figures 3 and 4). Madrone produced the longest sprouts when cut in February, but tanoak produced the longest sprouts when cut in April.

The number of sprouts per stump on treated and untreated stumps of the two species were analyzed by the chi-square method

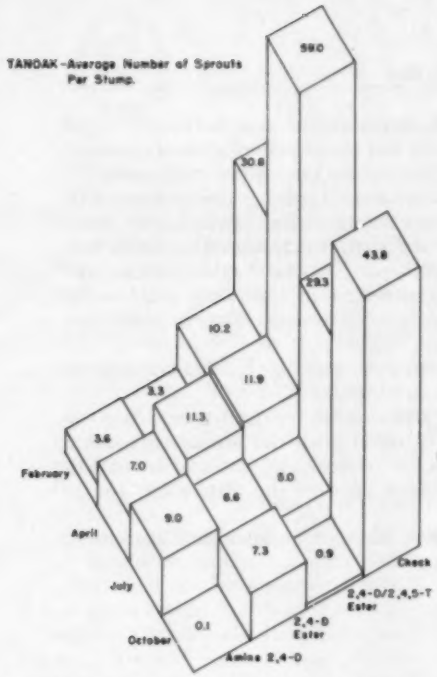

Figure 1. Tanoak—average number of sprouts per stump.

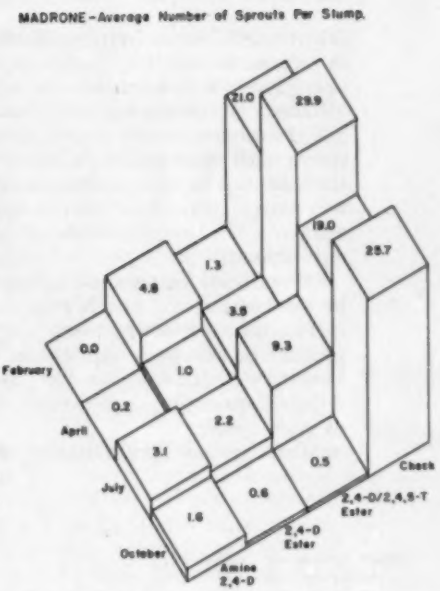

Figure 2. Madrone—average number of sprouts per stump.

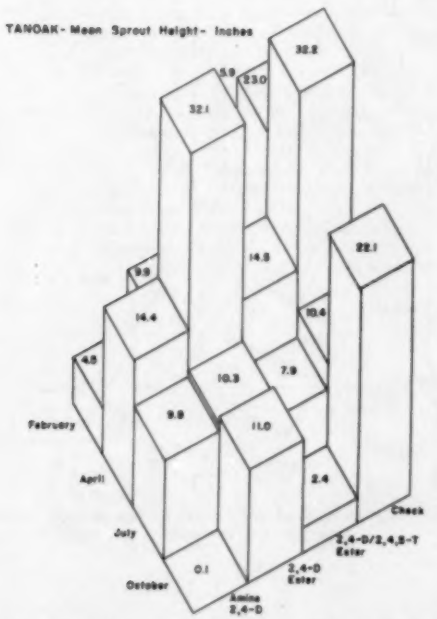

Figure 3. Tanoak—mean sprout height, inches.

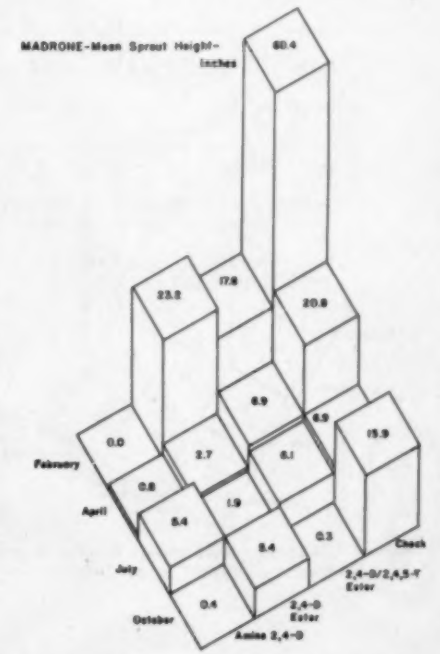

Figure 4. Madrone—mean sprout height, inches.

(2). All differences between dates of treatment and herbicides used in treatment were highly significant for both species with one exception. There was no difference between the two spray treatments. If all dates of treatment are consolidated, daubing the stump with 2,4–D amine reduced sprouting more than either of the spray treatments. All treatments reduced sprouting considerably below no treatment. The cost of the materials used in the daubing treatment was roughly one half the cost of materials in the spray treatments. Spraying is also more difficult because it requires more labor and equipment.

Of the four seasons tested, treating tanoak in October appears to be the best, giving nearly complete control.

The number of madrone sprouts can be reduced more than 98 percent in February, April, and October with the amine treatment. February treatment gave 100 percent control. All dates of treatment reduced sprouting considerably below that of the untreated stumps of each species.

Mean sprout height varied with date of cutting and treatment

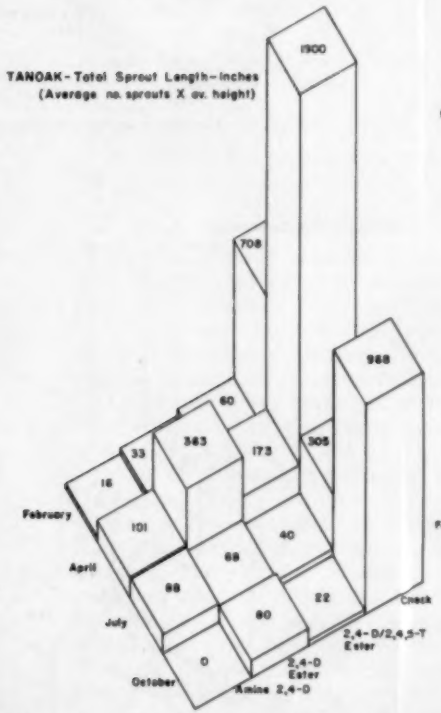

Figure 5. Tanoak—total sprout length, inches (average no. sprouts × av. height).

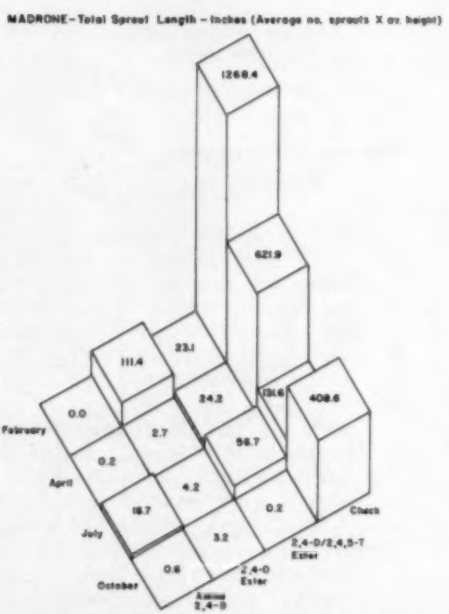

Figure 6. Madrone—total sprout length, inches (average no. sprouts × av. height).

used. With each species these variations closely resembled the variations in numbers of sprouts per stump. Control of sprout height of madrone was best from daubing 2,4–D amine on the cutting dates that produced the longest sprouts on untreated stumps (February, April, and October). Control of sprout height of either species was poorest from treatment in July when compared with the untreated stumps cut at the same date. This was also the month of the shortest sprout length on the untreated stumps of each species.

A volume figure that more nearly describes the sprouting condition as it appears in the field was determined by multiplying the average number of sprouts per stump by the average height of the sprouts. This gives a figure approximating the total length of sprouts produced per stump (Figures 5 and 6). This expression of data best shows the degree of actual control or reduction of the sprouting problem.

SUMMARY AND CONCLUSIONS

Daubing madrone and tanoak stumps with a 4 lb/gal ae solution of mixed alkanolamine salts of 2,4–D gave better control of sprouting than spraying stumps with 16 lb aehg solution of butoxy ethanol esters of either 2,4–D or 2,4–D + 2,4,5–T in diesel oil. Daubing the amine was also the least expensive treatment. Madrone was successfully treated in February, April, and October. Tanoak was best treated in February or October. The April treatment was poor for tanoak. July treatments gave poor results with both species.

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Absorption and Distribution of EPTC–S^{35}[1]

SHOGO YAMAGUCHI[2]

THE herbicide EPTC (ethyl *N,N*–di–*n*–propylthiolcarbamate) offers much promise as a soil-borne toxicant for controlling a variety of common weeds. Not only does it control annuals; it is proving very effective and quite specific in the control of nutsedge. In this role it is unique among the newer materials. In California it has held nutsedge in check during the whole growing season in strawberries, peppers, beans, and other row crops.

During the past season EPTC applied pre-emergence on corn and pre-plant on strawberries proved somewhat erratic. In a few instances there was crop injury; in many others there was no crop injury.

Selective action of EPTC is not well understood. Because it is more effective when incorporated into the moist top soil it seems that at least a part of its selectivity may be a positional effect. On the other hand, the general nature of its toxicity against many common weeds would seem to indicate a biochemical mechanism. If detoxification results from breakdown by an enzyme system of the plant, then a thorough understanding of the absorption, translocation, and ultimate fate of this chemical in the plant might clear up some of the uncertainty concerning its mechanism of action.

Ashton and Sheets (1) have demonstrated an inverse relationship of plant injury and adsorption of EPTC to soil. Fang and co-workers (3, 6) reported evidence of rather complete detoxification of the compound in various crop plants at maturity, from pre-emergence application. They have also presented data providing evidence for nearly complete loss or breakdown of EPTC in the red kidney bean during a 4 day period of germination following a 48 hour exposure to a 55 ppm solution. This breakdown did not occur in the mung bean.

The present work utilizes EPTC–S^{35}[3] and autoradiography and demonstrates absorption and distribution of EPTC in both susceptible and resistant plant species. Absorptional and distributional characteristics of EPTC were assessed from both foliage and root applications. By the counting method the ease of vapor movement of EPTC through the thickness of the bean leaf was assayed.

MATERIALS AND METHODS

Autoradiographic method for determination of distribution pattern.

The susceptible plants used included lima bean *(Phaseolus limensis),* jimsonweed *(Datura stramonium* L.), barnyardgrass *(Echinochloa crusgalli* (L.) Beauv.), and barley *(Hordeum vulgare).* The resistant plants used included red kidney bean *(Phaseolus vulgaris),* alfal-

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[3]One millicurie of sulfur–35–labeled EPTC was supplied by the Stauffer Chemical Company.

fa *(Medicago sativa)*, sugar beet *(Beta vulgaris)*, field bindweed *(Convolvulus arvensis)* and dent corn (*Zea Mays* var. indentata).

The seeds were planted in sand and transferred to solution culture in quart Mason jars, without forced aeration, and grown for several days. Application to leaves consisted of vapor and droplets of solution. Application to roots was through the culture solution. Applications were made mostly under a fume hood, but the treated plants were returned to the greenhouse. At the end of the treatment periods of 1 and 4 days, the plants were taken out of the jars and the roots blotted free of excess moisture. The plants were then spread out individually in a folded sheet of filter paper, 10 × 12 inches, pressed between a padded board and a piece of stiff hardware cloth held firmly in place with large stationery clamps, and open-air dried (8). Most plants dried in 3 days; sugar beet plants in 5 days. Our experience would indicate that the distribution pattern developed at the end of the treatment time was essentially retained intact by the drying method, except perhaps for the sugar beet plants of 1 day treatment time. The sugar beet plants showed unusually large movement of EPTC to the growing leaves for a 1 day treatment.

The dried pressed plants were placed against Kodak No Screen medical X-ray film, 10 × 12 inches, for a 4 weeks' exposure. The developed autoradiographs were backed with glossy white paper and examined.

The solution for root application was made up to 20 ml individually in quart Mason jars. EPTC can be dissolved in water by continued shaking; however, ready solution in culture solution was effected by a 3-step dilution (dilution to 5% with 70% ethyl alcohol; to ½% with 50% propylene glycol in water; and finally, with 0.1% of a commercial spray adjuvant containing alkylarylpolyoxyethylene glycols, free fatty acids and isopropanol (X–77) in tap water). The final 20 ml solution contained 470 micrograms of the labeled EPTC (23 ppm), 0.1% X–77, .035% ethyl alcohol and approximately 1.5 microcuries of S^{35}. The toxicity of a similar solution, without EPTC, was tested on field bindweed and was found to produce no adverse symptoms in 7 days. The toxicity of EPTC in this solution was tested at 50 ppm again on field bindweed and in 3 days necrosis began at shoot tips and leaf margins. The other species were not tested in this manner.

Also it was found that immediate solution of the technical grade (95%) EPTC in water can be accomplished by first incorporating EPTC in at least an equal volume of polyoxyethylene sorbitan monolaurate (Tween 20) and then delivering the desired amount of the resulting solution into the culture solution while stirring the latter. EPTC is released slowly and it has time to dissolve completely without forming droplets which rise to the surface.

The solution applied to the leaves contained water, 5% glucose, 55% ethyl alcohol and 1.18% EPTC. Forty microliters containing 470 micrograms of EPTC–S^{35} were applied per plant. Lanolin rings were used to confine the drops of solution. Contact injury was produced at the treated spot on most plants.

A more successful method of EPTC application to the leaves was the vapor method. In no instance was injury to leaves noticed, but the method was applicable only to the wider leaves, as of the red kidney and lima beans, sugar beet, and jimsonweed. Four hundred seventy micrograms with 1.5 microcuries, dissolved in 10 microliters of 70% ethyl alcohol were pipetted into a glass dish about a half inch across and placed on a leaf to be treated. Then a watch glass, several times wider, was inverted over the dish to confine the EPTC–S^{35} vapor to an area around the dish. A seal was effected with lanolin. Treatment time for all leaf applications was 4 days.

Counting method for determination of vapor movement through the leaf.

The autoradiographs of EPTC–S^{35} vapor treatment on bean plants and jimsonweed indicated the presence in the plants of only a small fraction of the applied radioactivity. A diffusional loss from the lower surface of the treated leaf was suspected. Therefore a method was devised to trap the diffusional loss and to assay the entrapped EPTC–S^{35} by counting with a thin window Geiger-Mueller tube.

The upper surface of the primary leaves of red kidney bean plants was given EPTC–S^{35} vapor application and from the lower surface of the same leaves, the escaping EPTC–S^{35} vapor was trapped with activated carbon (Norit A). The source of the vapor was a known amount of solution put in a glass dish about a half inch in diameter, placed over the area to be treated. The vapor was confined by an inverted shell vial one inch across and two inches deep and the seal was effected with lanolin. A one inch circle of fiber glass filter, sprayed with a suspension of 10 mg of activated carbon, was covered over with a piece of lens paper and then supported against the lower surface of the leaf, at the treated spot.

The dosage series used was 1, 5 and 10 microliters of a 70% ethyl alcohol solution containing 470 micrograms EPTC per 10 microliters. The total treatment time was 24 hours. The concentration of EPTC vapor at these dosages would have been approximately 250, 1250, and 2500 ppm (v/v) on the basis of complete vaporization of the applied dosage within the confinement of the inverted shell vial (22 ml capacity). The activated carbon pads were replaced with fresh ones at 2, 4 and 8 hours from the time of application of the solution. The experiment was duplicated. The pads were placed in cupped planchets and counted.

RESULTS AND DISCUSSION

The distribution pattern from drop applications to the leaves.

The autoradiographs of 4-day leaf treatments (both solution and vapor applications) showed two important translocation characteristics of EPTC: (1) the distribution pattern included growing stem tips and root tips and accumulation there in all species tested, indicating mobility of EPTC in phloem; and (2) the amount distributed in the plant from leaf application appeared to be but a small fraction of the applied amount.

In contrast to the growing regions, the mature green leaves generally showed no radioactivity. Some of the plants are shown in Fig. 1. Only in the field bindweed plant did the radioactivity show up faintly in mature leaves. Movement into mature leaves can occur by a transfer in the stem, from phloem to xylem, followed by transpirational movement into the leaves.

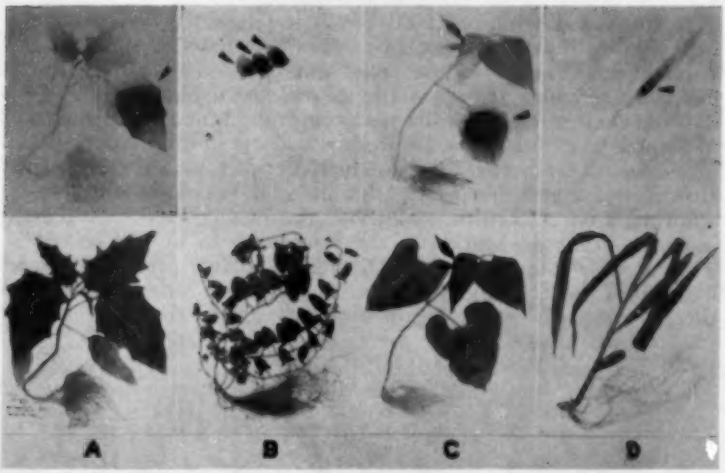

Figure 1. Distribution from foliar treatment with EPTC–S^{35}, 470 micrograms per plant, 4 days. The upper picture is the autoradiograph; the lower, the plant. The arrows point to the treated spot. A, Jimsonweed, vapor treatment. B, Field bindweed, solution treatment. C, Red kidney bean, vapor treatment. D, Corn, solution treatment.

The pattern of EPTC in the treated leaf itself indicated some apoplastic movement and a strong symplastic movement.

The apoplastic movement in the treated leaves (Figure 1) is the movement from the treated spot towards the tip of the treated leaf and does not include any basipetal movement. The reason for considering this sort of movement to be apoplastic is the fact that it occurs only in the direction of the transpiration stream, namely, toward the leaf tip or leaf margin, and not in the direction of assimilate movement, namely, toward the base of the leaf. In green leaves developed past the photosynthetic compensation point, the transpiration and assimilate movements are in opposite directions. Only such leaves were treated in Figure 1. Apoplastic movement has always been observed in the treated leaf when a mobile compound such as the diethanolamine salt of maleic hydrazide (2) is applied in a drop of solution near the base of the leaf blade.

A symplastic movement in Figure 1 is that movement from the treated spot to the growing regions, from "source to sink" with regard to the direction of assimilate movement. Only by being car-

ride in the symplastic movement, especially phloem movement, can there have been translocation from the treated mature green leaves to root tips, stem tips and enlarging leaves with the exclusion of translocation into other fully developed leaves. Note in Figure 1 that there is no trace of radioactivity in any of the mature green leaves other than the treated ones.

The distribution pattern from root application.

For root absorption of EPTC–S^{35} the treatment time was 1 and 4 days. The results of the 1-day treatment showed in 4 species a general distribution throughout the plant—in the roots, mature leaves, stems and growing regions, with the thicker tissues and the roots showing greater image density than the leaf lamina. There appeared to be no real difference of absorption and distribution from species to species. Differences of image density between the different plants are largely due to differences in the amount of retention of EPTC–S^{35} absorbed from the culture solution; the image density in general indicated ready absorption of the compound by all species tested. The leaves of the corn and barnyardgrass plants produced a light image, but these plants had begun to wilt a few hours after subjection to the treatment solution and only a part of the treatment solution was absorbed.

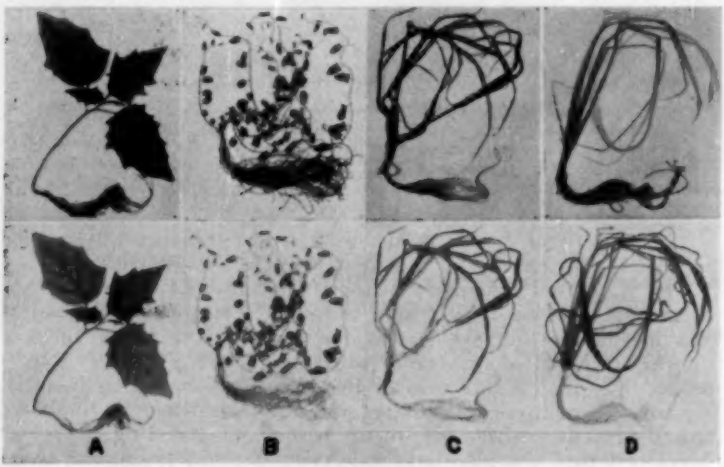

Figure 2. Distribution from root treatment with EPTC–S^{35}, 470 micrograms per plant, 4 days. The upper picture is the autoradiograph; the lower, the plant. A, Jimsonweed. B, Field bindweed. C, Barley. D, Barnyardgrass.

The autoradiographs of the 4-day treatment (Figure 2) showed an accumulation of the labeled material in all developing leaves, buds and root tips in addition to general distribution found with the 1-day treatment. This was well demonstrated in alfalfa, red kidney and

lima beans, jimsonweed, sugar beet and barley, but not in field bindweed. Corn and barnyardgrass plants wilted too much to show such results. The development of the accumulative effects in the regions of growth during the 4-day treatment is here explained as resulting essentially from a redistribution from initial uptake in mature leaves to the regions of growth. It involves a transfer of the compound from the apoplast to the phloem, if accumulative effects are to show up in the growing buds from root uptake.

Diffusional loss through the leaf.

Diffusional loss of EPTC vapor through the leaf was well demonstrated by capturing the escaping vapor on activated carbon. The activity recovered on activated carbon is assumed to be entirely of EPTC–S^{35}. Table 1 shows that the greatest diffusional losses were

Table 1. Counts per minute of activity (EPTC–S^{35}) recovered from the lower surface of the treated primary leaves of the red kidney bean plants, by absorption on activated charcoal.

Sampling time	Volume of treatment solution					
	1 µl (47 µg)		5 µl (235 µg)		10 µl (470 µg)	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
Total counts in the application	2300	2300	11,500	11,500	23,000	23,000
0–2 hr	1546	1928	578	968	1250	435
2–4 hr	52	46	1039	578	1952	994
4–8 hr	30	37	851	480	1777	748
8–24 hr	31	59	836	—	1832	1073
Total recovered counts	1659	2070	3300	2026	6811	3250
Percent activity recovered	72	90	29	(17% in 8 hr)	30	14

encountered at the lowest dosage, within the first two hours after application. These amounted approximately to 30 out of the 47 micrograms applied. The volume of the shell vial was 22 ml and the concentration of the EPTC–S^{35} vapor would have been no more than 250 ppm, v/v, over an area of one inch diameter. With the higher dosages the rate of initial diffusional loss was not increased, but actually decreased approximately to half and the loss was prolonged over days rather than hours. These rates would indicate, at least firstly, a movement through the gaseous phase (stomates and intercellular spaces) rather than through the solid and liquid phase (cell wall and protoplast). The higher dosages may have closed the stomates; however, no obvious leaf injuries developed from these treatments.

In this paper the results are presumed to indicate largely the movement of EPTC–S^{35}. The 4-day distribution pattern from root absorption was general. From leaf application the distribution was accumulative in the growing regions. From experience with other tracers (2, 3, 4) it seems unlikely that a compound which moves readily by xylem from root application could also move as freely by phloem from leaf application. Possibly some EPTC breakdown products are involved in the latter case (3, 6).

SUMMARY

The pattern of absorption, translocation, and distribution of EPTC shown by the autoradiographic method was similar in resistant and susceptible plant species. It was limited from leaf application of the vapor or solution and it was general from root application. Distribution from foliar application was to the growing tissues; distribution from root application was to all tissues, with greater accumulative effects showing in the growing tissues with the 4-day treatment. Some 72 to 90% of 47 μg EPTC–S^{35} vapor applied to an inch-diameter area of a bean leaf escaped in 24 hours from the opposite surface of the leaf, as measured by trapping in activated carbon. With larger dosages of vapor, the rate of escape was not greater, but only one-half as great.

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Effects of Surfactants on the Herbicidal Activity of Several Herbicides in Aqueous Spray Systems

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Introduction

Formulation and preparation of herbicidal chemicals for the selective control of weeds in mechanized crop production have become increasingly more difficult with the introduction of a wide variety of organic and inorganic herbicides during recent years. Commercial herbicides and experimental chemicals are usually applied in systems which contain not only the active ingredients but also various solvents, cosolvents, surfactants (surface-active agents), carriers, and other adjuvants. Formulations of herbicides are usually evaluated for their maximum effects on weeds and minimum effects on crop plants. The manner in which the inactive constituents of formulations influence the ultimate herbicidal activity of the active ingredients is not well understood and extensive research will be required to obtain the fundamental information needed.

Surfactants are perhaps the most universal constituents of herbicidal formulations. These chemicals possess manifold properties or characteristics which account for their employment in almost every phase of herbicide usage. In the synthesis, formulation, and mixing of herbicides, surfactants are added to provide emulsification, dispersion, or solubilization of various mixture components.

Surfactants affect the application of sprays, since they can change the viscosity of liquids. In preparing invert emulsions, for example, the properties imparted by surfactants influence the selection of delivery equipment. The importance of surfactants in differential wetting, spreading, and adherence of spray solutions on plant leaf surfaces has been recognized since the turn of the present century.

Early research on the role of surfactants in herbicidal formulations was primarily concerned with their packaging, marketing, and efficient application. Little attention was given to differential effects of surfactants on herbicidal activity, although investigators progressively realized that the most effective surfactant for use in formulations of a chemical such as 2,4–dichlorophenoxyacetic acid [2,4–D] for the control of annual broadleaved weeds in cereals might not possess the desired properties in formulations of other chemicals or of 2,4–D for the control of weeds on non-crop land.

Surfactant actions in the external environment of plants have been specifically and critically studied in the past. Knowledge of internal surfactant effects on penetration, translocation, and herbi-

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cidal activity, however, is limited. Because of the diversified properties which an individual surfactant may possess, comparative investigation of the aggregate effects of a large number of surfactants on the selective action of several herbicides appeared desirable.

In 1958, investigations of the effects of surfactants on herbicidal activity were begun at the Plant Industry Station of the U. S. Department of Agriculture at Beltsville, Maryland. The immediate objectives were (a) to develop methods for comparative evaluation of surfactant effects on the activity of several herbicides, (b) to compare a large number of surfactants by such standardized evaluation procedures, (c) to study the relative effectiveness of surfactant-herbicide combinations in relation to their structures and physical and chemical properties, and (d) to develop principles for the selection and incorporation of surfactants in herbicidal formulations. The present report contains the preliminary evaluations of surfactant effects in aqueous herbicidal spray systems.

REVIEW OF LITERATURE

No attempt will be made to review the rather voluminous literature on the multiple uses of surfactants. The problems and principles involved in the addition of surfactants in formulating and mixing agricultural pesticides are common to many other fields of the chemical industry, such as the formulation, handling, and dispensing of pharmaceuticals, cosmetics, and petroleum products, as well as many other non-pesticidal agricultural chemicals. Most biological studies of surfactant influences on pesticidal action have been conducted in field trials with commercial surface-active agents (12). Accurate interpretation of the results of such studies has been difficult because of a lack of information on structure and purity of the surfactants.

An early report on the use of surface-active agents in 1890 showed that the application of arsenical insecticides in strong soapy solutions or in flour-paste suspensions increased insecticide damage to foliage, whereas application in a saponified resin solution was no more damaging than in water alone (16). Inasmuch as phytotoxicity of insecticidal sprays was considered a detrimental characteristic, the full significance of this early work on herbicidal action was little appreciated. However, the fact that the efficiency of insecticidal sprays could be improved by proper formulation without increasing foliar damage was quickly exploited in succeeding years in the formulation of pesticides for the control of insects and diseases.

The physical forces operating at interfaces and the free-energy characteristics of surfactant solutions have received the attention of investigators for more than 75 years. Major advances with respect to pesticidal sprays were reviewed critically in 1921 (23) and in 1959 (12). In 1915, the contact angle was introduced as a physical quantity which provided a vectoral representation of the interplay between several surface forces (3). Expansion of the contact-angle concept permitted distinction of the phenomena of wetting and

spreading (24). Later a refined quantity, the spreading coefficient, was developed (29). However, no single physical quantity proved adequate for indexing the wetting and spreading characteristics of pesticidal sprays. The importance of vital forces in penetration of a pesticide was emphasized by the discovery that penetration of soap solutions through the trachea of an insect could not be completely accounted for by consideration of either capillary forces or the contact angle. No penetration of the solution occurred if the insect larva had been previously killed with potassium cyanide (29).

Before the introduction of synthetic organic herbicides in the mid-1930's, little attention was given to the importance of spray composition in foliar penetration. Earlier workers had been interested primarily in achieving adequate coverage and chemical deposition of contact insecticides and fungicides. In contrast, herbicides must also penetrate living and non-living plant tissues. Nine causes have been given for the increased herbicidal effectiveness which may result from the use of surfactants (6). However, evidence to establish the relative contribution of each to the aggregate penetration-activity process is critically needed.

Some surfactants were shown to increase spreading on waxy or non-waxy surfaces but not necessarily on both (23). Critical studies demonstrated that selective phytotoxicity is partly a function of differential spray retention by leaves of readily wettable and non-wettable species (1, 2). On species which were difficult to wet retention was a linear function of spray volume with characteristic slopes for different surface tensions. On wettable leaves, however, retention was independent of surface tension at low volumes and could not be increased above a plateau at a low surface tension. Another study revealed that the electro-negativity of the leaf surface in relation to the form in which a chemical was incorporated into the spray could materially affect the final chemical deposit (23).

Surfactant interactions with herbicidal chemicals and enhancement of herbicidal activities were reported in the earliest days of experimentation with 2,4–D (31). Through the addition of surfactants, several investigators later found significant increases in activity of a number of herbicides and growth regulators applied as sprays and dusts (10, 17, 18, 19, 20, 27, 28, 30). Such enhancement must be partly attributed to increases in rate or quantity of chemical uptake (11, 21, 22). The magnitude of increased uptake appears to depend on the nature of the surfactant and the concentration and form of chemical (18, 22, 25). Few investigators studied the influence of surfactant concentration and seldom employed the same surfactants in conducting work with different herbicides. The preceding facts perhaps help to resolve apparently conflicting reports of surfactant effects found in the literature (5, 27).

Reports of inherent phytotoxicity of surfactant solutions are as old as the history of surfactant additions to pesticidal sprays. Selective phytotoxicity of soapy solutions was reported in 1890 (16). Similar toxicities of sulfated alcohols were reported in the mid-

1930's (4, 8). More recently, the toxicity of 39 proprietary surfactants to roots and leaves of six species was determined (15). However, many of the materials were of a mixed composition, and further determination of the phytotoxic principles would be very difficult. In an investigation of a homologous series of fatty acids and their potassium soaps, phytotoxicity was correlated with the length of the alkyl chain (7). Phytotoxicity of the fatty acids to tomato, tobacco, potato, bean, cabbage, and nasturtium was found to be associated with the C–6 to C–12 members of the series. Among the soaps of these fatty acids, however, the phytotoxicity was largely restricted to the C–6 moiety. The concentration of either the acid or the soap determined the magnitude of phytotoxicity. In more recent investigations other factors reported to influence the degree of surfactant phytotoxicity to apple and plum leaves were found to be the age of the leaf, the variety of the species, nutritional status, weather, (13) and a number of physico-chemical factors (14). The interaction of the surfactant phytotoxicity with herbicidal phytotoxicity has not been the subject of any definitive investigation in the past.

In summation, surfactants influence a number of aspects of herbicidal spray behavior. The influences are exerted in the formulation of herbicidal concentrates, in the mixing of the concentrates in the spray tank, in spray delivery, in surface deposition, and to a certain extent in penetration. Definitive knowledge of the aggregate effects of surfactants on the activity of herbicides has been derived from widely separated sources which have no common bases for comparisons. The present work attempts to index the aggregate effects by standardized procedures. Preliminary comparative evaluations of a large number of surfactants with several herbicides and on two representative species have been conducted.

MATERIALS AND METHODS

Corn (*Zea mays* cv. US 13) and soybean (*Glycine max* cv. Clark) were used throughout these studies as representatives of monocotyledonous and dicotyledonous species and of plants with waxy and hairy leaf surfaces, respectively. Seeds were germinated and plants were grown under greenhouse conditions in polystyrene containers containing high-fertility potting soil of pH 6.5. Plants were thinned for uniformity to 2 plants per pot after emergence. Sprays were applied to both species just after expansion of the first trifoliate leaf of soybean, at which time the corn was in the 4- to 5-leaf stage. Plants were grown for two weeks after treatment. Moisture levels for optimal plant growth were maintained by sub-irrigation.

Herbicides of a high degree of purity without additives of any type were employed. Those selected as representative of major classes were as follows: sodium salt of 2,2–dichloropropionic acid [dalapon]; triethanolamine salt of 2,4–D, prepared from technical grade 2,4–D and pure triethanolamine; triethanolamine salt of 4,6–dinitro–*o*–*sec*–butylphenol [DNBP], prepared from technical DNBP and

pure triethanolamine; and 3–amino–1,2,4–triazole [amitrol].[2] All herbicides were water soluble.

The 63 surfactants included in these studies were selected as representatives of the nonionic, anionic, cationic, and ampholytic classes of surfactants. Several blended surfactants were also included. These surfactants were selected from the accumulated stocks of proprietary and experimental surfactants on the basis of available purity information and physical or chemical data. Surfactants are referred to by accession number throughout the following pages. Pertinent chemical information, proprietary designations, and sources of the various surfactants are given in Table 1. Surfactant number S–145, a nonionic polyoxyethylene sorbitan monolaurate, was selected as a standard for comparative studies and was employed in all experiments.

Experimental treatments consisted of the individually formulated herbicide–surfactant systems. Surfactants in the first ten experiments were evaluated at concentrations of 0.001, 0.01, 0.1, and 1.0 percent on a weight-per-volume basis.[3] In later experiments the 0.001-percent concentration was omitted. Each treatment was replicated three times in a randomized-block experimental design. Appropriate herbicide and surfactant check treatments were included in each experiment.

Applications were made by means of an endless belt spray table. In one experiment, spray volumes of 5, 10, 20, and 40 gal/A were applied. Uniform conditions of spray application were maintained by using a single nozzle tip and constant pressure. The different volume delivery rates were achieved by varying the speed of the spray table and the height of the nozzle. Only the 40-gal/A volume was employed in other experiments.

Experimental results are reported in terms of an activity index derived from 4 evaluations of herbicidal effectiveness. Seven days after treatment a *contact injury rating* was assigned visually in accordance with the following scale: 0—no injury, 1–3—varying degrees of spotting and minor leaf burn, 4–6—moderate degrees of burn or defoliation, 7–9—severe leaf burn or severe reduction of foliage, 10—complete killing of the test plants. A *general injury rating*, based upon a similar 0–10 scale for slight, moderate, and severe injuries, ascertained from morphogenic effects and general physiognomic characteristics, was assigned 14 days after treatment. Randomly selected untreated groups of test plants were measured and weighed at the beginning of each experiment for comparative determination of treatment effects on height and weight during the 2-week treatment period. All *height data* were based upon individual measurements and all *weight data* upon the pooled top weights per pot. Growth data of plants in water-sprayed check treatments were used as reference standards for calculating percentage reductions in height and weight in other treatments. The average of these four values—con-

[2]Pictorial records were made before the approval of "amitrole" as the common name for 3–amino–1,2,4–triazole. The earlier name "amitrol" is therefore retained throughout the present paper to provide for uniformity in usage.

[3]Grams of active surfactant per 100 ml of solution.

tact injury rating, general injury rating, and height and weight reduction percentages—on a 0 to 100 basis constitutes the activity index, in which 0 represents no injury and 100 indicates death of the test plants.

The experiments reported herein were conducted under ambient greenhouse conditions during September to June of the seasons starting in 1958 and 1959. Maximum day temperatures averaged about 90° F. but ranged from 70°–110° for the coldest and hottest periods, respectively. Night temperatures averaged 10°–15° lower than the daily maxima but through use of thermostatically controlled heaters were not permitted to fall below 70°. Photoperiods of 14–15 hours were maintained by supplemental incandescent lights during the winter.

RESULTS

Standardization of evaluation techniques.

Two preliminary experiments were conducted to develop adequate and convenient experimental techniques and procedures. In the second experiment the standard surfactant, S–145, was evaluated in combination with dalapon at ½, 1, and 2 lb/A; 2,4–D at $^1/_{32}$, $^1/_{16}$, and ⅛ lb/A; DNBP at ¼, ½, and 1 lb/A; and amitrol at ¼, ½ and 1 lb/A. Increases in surfactant concentration characteristically increased the degree of herbicidal response of one or both species to each of the individual rates of all herbicides. Typical experimental results are depicted in Figure 1, which illustrates the effects of 2,4–D and amitrol on soybean.

Several important conclusions concerning surfactant action in herbicidal sprays may be drawn from the results of the second experiment. (1). Herbicidal activity was a function of both the rate of herbicide application and the concentration of surfactant in the spray solution. In factorial arrayments of the treated plants (Figure 1) levels of herbicidal activity could be contoured. (2). The S–145 standard surfactant possessed little or no phytotoxicity to the test species over the range of concentrations employed. (3). The magnitude of surfactant enhancement of activity at a given herbicide level was increased as the rate of application of herbicide was decreased.

On the basis of the results of the preliminary experiments, rates of each herbicide were selected for inclusion in subsequent evaluation experiments as follows: for dalapon, 1 lb/A; for 2,4–D, $^1/_{16}$ lb/A; for DNBP, ½ lb/A; and for amitrol, ¼ lb/A.

Effect of volume rate of spray application.

Although the preliminary experiments permitted the standardization of procedures for systematic evaluation of surfactants, they did not establish whether the surfactant effects obtained were a function (a) of the per-unit-volume concentration of the surfactant or (b) of the actual amounts of surfactant received by each plant. The importance of these two possibilities was investigated in an experiment in which the standardized rates of herbicide application and the per-unit-volume concentrations of surfactant were held con-

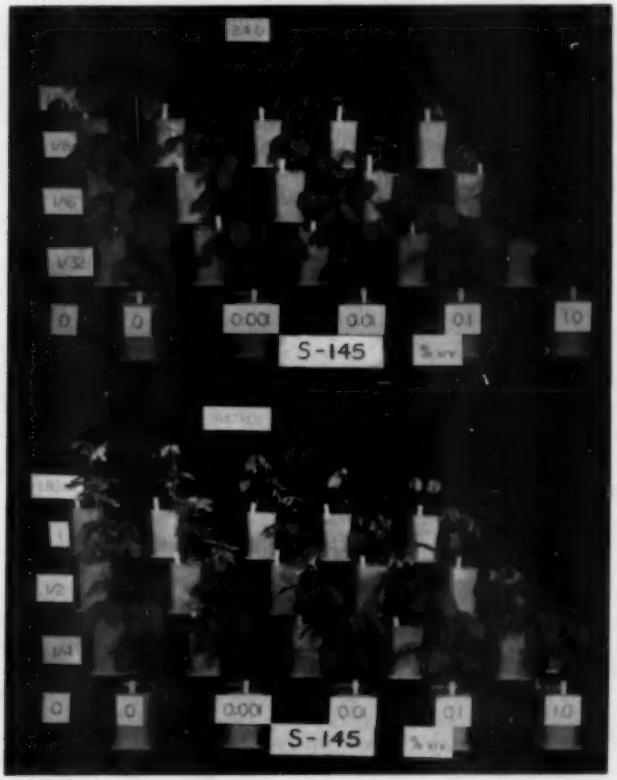

Figure 1. Relation of herbicide rate and surfactant concentration to activity of herbicides on soybean: top, 2,4–D; bottom, amitrol.

stant. The various herbicide–surfactant combinations were applied in spray volumes of 5, 10, 20, and 40 gal/A. Typical results, showing the activity indices for dalapon- and 2,4–D-treatments on the two test species, are illustrated in Figure 2.

Interpretations of the results of this experiment are based upon the following considerations. If the per-unit-volume concentration of surfactant is the important point, the relative enhancement of herbicidal activity should remain relatively constant at any one surfactant concentration regardless of spray volume. On the other hand, if the amount of surfactant is the important consideration, proportional decreases in surfactant enhancement of herbicidal activity should be experienced as spray volume is decreased from 40 to 5 gal/A at any one surfactant concentration. At the 5-gal/A rate, the amount of surfactant is reduced to 1/8 of the amount applied at 40 gal/A. At the same time, the amount of surfactant applied at one

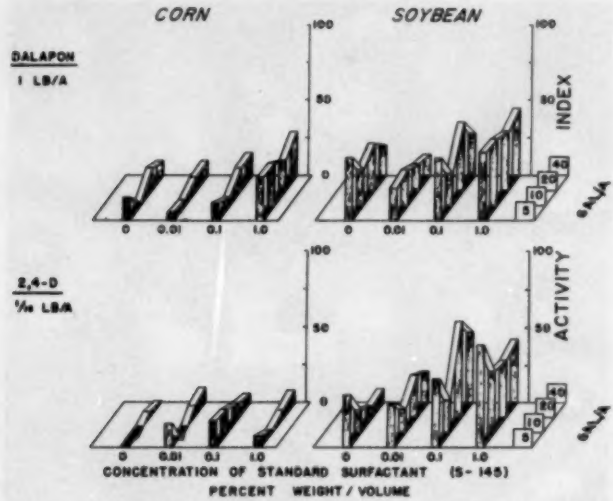

Figure 2. Effects of spray volume and surfactant concentration on herbicidal activity of dalapon and 2,4-D on corn and soybean.

surfactant concentration is $^1/_{10}$ of that at the next higher concentration at a given spray volume. Therefore, a closer agreement should exist between the 5-gal/A value at the 1.0-percent surfactant concentration and the 40-gal/A rate at the 0.1-percent concentration than between the 5- and 40-gal volumes of the 1.0-percent concentration. Figure 2 reveals that the experimental results fit better the interpretation that surfactant concentration, not the amount, is the important consideration. Similar results were obained with DNBP and amitrol.

A nonionic polyoxyethylene thioether [S–102], which had shown considerable phytotoxicity per se at the 1.0-percent concentration in other experiments, was also included in the concentration-volume experiment at the 1.0- and 0.1-percent concentrations. In other experiments, phytotoxicities due to surfactants had been found to be generally additive to the typical surfactant enhancement such as is attained with S–145. The purpose for including S–102 was to determine whether decreases in the volume rates of application would influence this phytotoxicity. Results indicated that as spray volume was lowered and the amount of surfactant decreased, surfactant phytotoxicity was also reduced. Herbicidal activity responses more nearly approached those of the standard surfactant combinations. In general, then, except for instances of inherent surfactant phytotoxicity, the per-unit-volume concentration of the surfactant exerted the greater influence on surfactant enhancement of herbicide activity.

Statistical evaluation of standard evaluation treatments and effects of the standard surfactant on herbicidal activity.

The inclusion of the standard-surfactant and herbicide-check treatments in each evaluation experiment permitted verification of the usefulness of techniques by certain statistical analyses of data. The means and standard errors for these treatments from the first ten evaluation experiments are plotted in Figure 3. An analysis of variance for the activity indices showed that all differences due to herbicides, concentrations, species, interactions, and replications in time were highly significant but that least variance was to be expected from replication of the treatments in time. The calculated LSD's for comparison of means were as follows: $P_5\% = 6.1$, $P_1\% =$

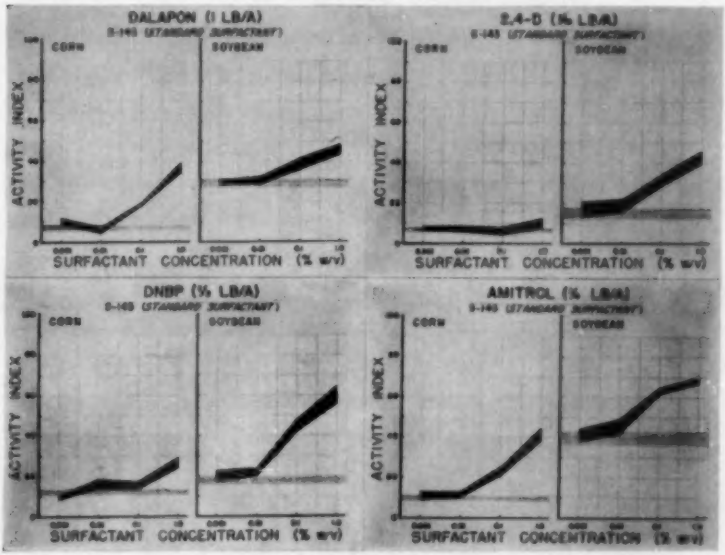

Figure 3. Effects of a polyoxyethylene sorbitan monolaurate [S–145] on the activity of four herbicides on two species.—Light horizontal bars denote activity of each herbicide in the absence of surfactant. Dark curves are plotted for the surfactant-herbicide combination treatments. Width of bars represents the mean ± the standard error, calculated from data from 10 experiments.

8.0. The coefficient of variability was 10.6%. The validity of experimental results would seem to be adequate on the basis of these statistics.

With but two exceptions, surfactant S–145 showed a characteristic enhancement of herbicidal activity over the entire range of surfactant concentrations used. Highly significant increases in herbicidal activity were obtained at concentrations of surfactant greater than a threshold concentration of 0.01 percent. Each curve shows a more or less linear relationship between activity index and the

Table 1. Activity indices on corn and soybeans for four herbicides in combination with several concentrations of various surfactants. Averages of four measurements of treatment effectiveness: 100, complete killing of treated plants; 0, no effect; negative values, growth stimulation.

Surfactant number	Chemical description [Proprietary name] (Source)[a,b]	Conc. (% w/v)	Corn					Soybeans				
			Water	Dalapon 1 lb/A	2,4-D ⅛ lb/A	DNBP ⅓ lb/A	Amitrol ¼ lb/A	Water	Dalapon 1 lb/A	2,4-D ⅛ lb/A	DNBP ⅓ lb/A	Amitrol ¼ lb/A
REFERENCE TREATMENTS												
	No surfactant	0	0	7	6	12	10	0	29	15	18	40
S-145 (non-ionic)	polyoxyethylene sorbitan monolaurate [Tween 20] (7)	0.001	0	10	7	10	11	0	30	17	20	41
		0.010	3	6	7	16	12	1	31	19	22	45
		0.100	3	18	6	15	23	4	39	31	45	63
		1.000	4	36	10	28	41	5	46	42	60	68
NONIONIC SURFACTANTS												
S-018	polyoxyethylene red oil amide [Ethomid 0/15] (4)	0.010	−6	8	−2	7	20	0	27	−1	34	38
		0.100	0	12	7	18	21	6	30	6	55	50
		1.000	14	25	10	28	40	14	41	22	83	57
S-024	polyoxyethylene glycol ester of coco fatty acids [Ethofat C/25] (4)	0.001	3	6	6	18	8	−1	14	7	22	52
		0.010	5	11	12	18	16	3	25	6	33	47
		0.100	10	33	7	22	33	2	22	28	63	57
		1.000	10	34	6	56	48	3	29	38	76	66
S-032	[AHCO Base Oil 90] (5)	0.010	−5	6	4	11	16	13	11	−7	28	31
		0.100	4	0	−4	12	10	8	33	−4	58	32
		1.000	5	28	1	23	30	4	34	8	93	50
S-037	polyalkylene glycol ether [Tergitol Nonionic XD] (17)	0.010	0	2	4	6	17	1	26	23	32	38
		0.100	3	17	10	44	22	10	33	29	51	47
		1.000	0	24	7	5	52	22	49	44	51	60
S-038	alkylphenyl ether of polyethylene glycol [Tergitol Nonionic NPX] (17)	0.001	6	6	−9	6	8	2	37	21	18	56
		0.010	3	14	−1	6	−2	2	38	25	34	58
		0.100	9	28	4	8	36	5	46	53	45	68
		1.000	10	55	36	58	63	26	63	63	73	73
S-039	trimethyl nonyl ether of polyethylene glycol [Tergitol Nonionic TMN] (17)	0.001	6	11	10	22	9	−8	12	3	21	44
		0.010	2	18	9	17	9	−6	15	5	26	59
		0.100	−1	33	12	44	41	3	37	32	66	61
		1.000	38	58	43	100	74	30	58	55	92	72

S-050	glyceryl monococate-C (8)	0.010	3	6	2	10	16	6	24	11	21	39
		0.100	1	12	6	20	18	−6	13	25	46	46
		1.000	4	35	1	61	38	7	37	16	74	43
S-051	lauryl-myristyl diethanolamide (8)	0.010	3	11	14	11	15	5	32	16	28	45
		0.100	9	16	0	10	20	13	26	15	47	51
		1.000	1	29	12	39	38	21	46	36	62	65
S-054	propylene glycol monococate-C (8)	0.010	6	2	9	10	2	4	14	10	17	28
		0.100	8	16	−1	16	15	12	17	13	19	30
		1.000	−9	10	2	19	2	9	23	4	43	23
S-100	polyoxyethylene ester of tall oil [Sterox CD] (14)	0.001	9	16	8	4	10	−6	36	28	24	38
		0.010	4	9	8	−1	0	−4	32	27	16	43
		0.100	−8	18	3	6	14	−6	37	32	22	57
		1.000	6	26	−2	16	14	4	50	36	53	59
S-102	polyoxyethylene thioether [Sterox SK] (14)	0.001	11	5	5	13	12	−5	30	−1	13	50
		0.010	17	14	12	19	21	−4	33	6	25	50
		0.100	3	20	0	23	30	−4	36	26	73	65
		1.000	36	56	34	91	64	30	54	58	92	76
S-103	aliphatic polyoxyethylene ether type [Sterox A]] (14)	0.010	−4	0	11	3	14	9	26	17	33	40
		0.100	0	15	6	16	42	6	34	30	52	46
		1.000	45	56	48	83	29	30	54	46	77	53
S-111	alkylphenol ethylene oxide condensate type [KYRO EO] (15)	0.010	6	14	10	5	18	0	23	19	27	54
		0.100	3	12	11	7	15	10	38	28	36	72
		1.000	20	53	8	10	33	18	60	56	56	75
S-113	alkyl aryl polyether alcohol [Triton X-100] (16)	0.001	4	9	8	15	21	−9	35	4	18	46
		0.010	2	16	17	18	18	−8	32	7	28	51
		0.100	14	31	7	18	35	1	41	43	46	64
		1.000	29	52	29	26	58	29	57	63	88	83
S-115	alkyl aryl polyether alcohol [Triton X-114] (16)	0.001	−2	10	2	16	−2	1	35	19	20	51
		0.010	8	9	4	8	18	−3	32	28	34	58
		0.100	2	12	0	4	18	13	44	46	34	62
		1.000	23	67	35	60	68	30	58	72	78	74
S-116	alkyl aryl polyethoxy ethanol [Triton X-45] (16)	0.010	−2	8	19	16	13	7	30	12	27	50
		0.100	9	21	1	22	24	5	38	14	32	48
		1.000	11	22	7	41	36	14	39	33	52	59
S-120	alkyl phenoxy polyethoxy ethanol [Triton X-155] (16)	0.001	2	12	10	16	11	−4	33	14	19	49
		0.010	3	10	10	18	31	−8	32	10	25	54
		0.100	5	15	5	8	31	−2	33	10	38	57
		1.000	18	34	16	25	53	8	34	44	63	72

Surfactant number	Chemical description [Proprietary name] (Source)[a,b]	Conc. (% w/v)	Corn					Soybeans				
			Water	Dalapon 1 lb/A	2,4-D ⅛ lb/A	DNBP ½ lb/A	Amitrol ¼ lb/A	Water	Dalapon 1 lb/A	2,4-D ⅛ lb/A	DNBP ½ lb/A	Amitrol ¼ lb/A
S-123	modified phthalic glycerol alkyl resin [Triton—B-1956] (16)	0.010	−12	4	6	10	10	9	40	13	26	58
		0.100	1	16	3	14	16	14	26	12	29	52
		1.000	13	10	2	20	23	11	20	16	33	58
S-126	alkyl aryl polyether [Triton CF-10] (16)	0.010	−6	6	9	13	8	9	36	10	29	58
		0.100	4	8	1	26	22	14	45	28	38	68
		1.000	[illegible]	35	16	12	43	20	44	54	58	80
S-127	alkyl aryl polyether alcohol [Triton X-102] (16)	0.010	6	12	2	16	2	6	33	20	18	59
		0.100	4	18	0	16	16	7	40	28	26	75
		1.000	23	34	5	7	46	24	57	46	44	76
S-137	polyoxyethylene polyoxypropylene [Pluronic L64] (19)	0.001	1	5	13	20	10	−8	16	2	10	52
		0.010	−3	2	15	18	16	−4	18	18	25	61
		0.100	3	23	10	19	39	2	30	22	51	62
		1.000	10	36	6	50	41	16	44	44	62	68
S-140	(polyoxyethylene) (polyoxypropylene) ethylenediamine [Tetronic 908] (19)	0.001	13	16	15	12	20	−6	26	12	27	58
		0.010	8	11	16	21	6	−4	30	17	29	62
		0.100	14	16	15	16	20	5	24	18	40	62
		1.000	12	35	10	30	52	2	38	48	66	71
S-154	nonyl phenoxy polyoxyethylene ethanol [Igepal CO-630] (3)	0.001	8	13	12	12	5	3	27	11	22	27
		0.010	4	22	8	18	13	2	36	13	21	36
		0.100	12	22	14	17	5	8	45	27	26	64
		1.000	12	30	19	18	23	20	51	43	60	68
S-158	polyoxyethylene glycol ether of nonylphenol [AF 100] (13)	0.010	−2	6	4	16	8	6	16	8	18	30
		0.100	−6	14	8	12	20	7	24	14	28	50
		1.000	16	33	20	20	50	20	44	45	50	63
ANIONIC SURFACTANTS												
S-009	sodium dihexyl sulfosuccinate [Aerosol MA 80%] (2)	0.010	3	11	2	9	27	12	32	4	38	38
		0.100	5	16	−1	13	20	10	36	25	62	51
		1.000	20	27	4	80	49	12	50	26	90	68
S-012	sodium dioctyl sulfosuccinate [Aerosol OT-B] (2)	0.010	−6	0	−2	10	11	8	26	2	43	40
		0.100	6	13	3	13	21	12	37	16	69	53
		1.000	16	34	16	44	42	28	66	40	87	86
S-013	N-octadecyl disodium sulfosuccinamate [Aerosol 18] (2)	0.010	6	6	16	11	11	−7	25	−2	29	36
		0.100	2	8	5	8	10	6	30	−6	30	46
		1.000	−1	10	0	8	6	13	42	1	34	43

S–017		0.001	6	–3	6	19	5	4	27	24	13	40
	water-soluble acrylic polymer	0.010	10	5	6	14	19	7	38	29	13	43
	(hydrolyzed polyacrylonitrile)	0.100	6	16	6	13	6	6	15	25	13	45
	[Cyanamer 370] (2)	1.000	16	10	26	10	0	11	49	33	23	51
S–034		0.001	–4	0	1	4	0	–12	28	14	19	45
	alkyl aryl sulfonate type, sodium salt	0.010	4	17	8	16	14	7	25	22	34	50
	[Ultrawet "K"] (6)	0.100	–4	18	3	18	12	3	38	19	53	60
		1.000	15	43	21	83	51	9	50	32	58	68
S–035		0.001	–4	18	–4	6	1	13	34	26	18	36
	alkyl aryl sulfonate type, sodium salt	0.010	0	0	5	9	6	6	32	19	34	39
	[Ultrawet "DS"] (6)	0.100	–7	10	–10	11	6	8	35	16	45	56
		1.000	5	44	12	44	33	10	49	39	43	70
S–036		0.001	6	4	0	–1	10	0	31	9	6	29
	alkyl aryl sulfonate type, amine salt	0.010	8	–2	1	7	12	4	26	14	10	26
	[Ultrawet 60L] (6)	0.100	–5	8	6	9	4	1	29	9	16	30
		1.000	15	18	12	13	19	3	29	18	34	56
S–052		0.010	10	13	12	1	13	1	25	14	39	42
	sodium dodecylbenzene sulfonate (8)	0.100	8	17	2	12	17	0	27	10	42	46
		1.000	16	48	10	65	46	16	52	27	71	55
S–053		0.010	8	3	9	10	11	0	24	10	29	48
	sodium H-coco monoglyceride sulfate	0.100	5	12	5	20	18	6	28	13	49	46
	(8)	1.000	7	20	11	16	34	18	41	30	60	48
S–061												
	sodium salts of polymerized substituted	0.010	6	8	4	2	9	12	24	20	26	38
	benzoid alkyl sulfonic acids	0.100	5	17	2	4	4	8	27	14	22	35
	[Daxad 23] (10)	1.000	2	11	0	1	17	0	22	6	24	40
S–062												
	sodium salts of polymerized alkyl	0.010	6	14	6	6	–2	–5	22	9	13	18
	naphthane sulfonic acids	0.100	12	8	7	10	5	18	11	8	14	28
	[Daxad 11] (10)	1.000	13	6	9	7	5	10	11	12	14	24
S–063												
	sodium salts of polymerized substituted	0.010	–3	7	12	20	–2	0	14	12	18	17
	benzoid alkyl sulfonic acids	0.100	–1	5	0	16	4	12	18	11	19	32
	[Daxad 27] (10)	1.000	2	7	–1	5	8	8	12	5	15	17
S–064		0.001	6	6	6	1	9	0	24	12	24	36
	mono-calcium salt of polymerized aryl	0.010	–1	9	4	14	4	1	29	16	20	46
	alkyl sulfonic acids [Daxad 21] (10)	0.100	–4	15	4	13	7	–2	32	9	26	39
		1.000	0	9	8	8	–2	–3	19	12	19	33

Surfactant number	Chemical description [Proprietary name] (Source)[a,b]	Conc. (% w/v)	Corn					Soybeans				
			Water	Dalapon 1 lb/A	2,4-D ¼ lb/A	DNBP ½ lb/A	Amitrol ¼ lb/A	Water	Dalapon 1 lb/A	2,4-D ¼ lb/A	DNBP ½ lb/A	Amitrol ¼ lb/A
S-076	sodium lauryl sulfate [Duponol WAQ] (11)	0.001	4	14	16	20	21	4	28	11	22	60
		0.010	6	25	3	15	25	−2	21	16	34	62
		0.100	21	34	13	28	35	9	32	12	55	60
		1.000	18	60	25	38	51	6	41	40	64	64
S-081	amine salt of alkyl aryl sulfonic acid [Emcol H-86C] (12)	0.001	3	18	2	3	0	2	42	39	33	36
		0.010	0	2	6	14	12	8	38	32	32	58
		0.100	1	4	7	34	10	−2	49	44	50	61
		1.000	22	74	38	95	45	17	65	63	63	73
S-098	alkyl aryl sulfonate, specially processed [Santomerse SX] (14)	0.001	4	11	7	12	7	4	34	22	18	40
		0.010	7	15	7	9	16	1	36	13	33	46
		0.100	8	19	2	10	16	−8	41	15	40	58
		1.000	9	26	14	52	41	14	46	30	56	65
S-099	alkyl aryl sulfonate [Santomerse 8] (14)	0.001	−7	2	−18	14	8	7	30	15	25	39
		0.010	−6	0	12	12	10	9	35	18	22	42
		0.100	0	15	4	38	21	1	39	13	46	55
		1.000	4	25	18	47	38	15	50	49	43	64
S-101	alkyl aryl sulfonate [Santomerse No. 80] (14)	0.001	1	15	0	6	−1	−6	29	14	12	24
		0.010	2	−1	6	10	2	−2	28	19	14	29
		0.100	−2	−2	1	8	11	3	40	9	18	47
		1.000	17	20	14	23	14	0	40	26	35	54
S-110	sodium alkyl aryl sulfonate type [Orvus AB Granules] (15)	0.010	0	5	1	5	21	4	24	13	31	44
		0.100	8	8	2	17	31	4	34	18	66	52
		1.000	10	41	9	75	77	20	42	28	50	67
S-117	sulfonated alkyl ester [Triton GR-7] (16)	0.010	−6	11	2	8	8	2	33	15	22	48
		0.100	1	15	4	21	10	9	40	21	38	59
		1.000	28	40	41	80	34	20	56	53	74	71
S-125	sodium salt of an alkyl aryl polyether sulfonate [Triton X-200] (16)	0.010	−3	−4	12	22	18	4	27	17	16	64
		0.100	1	20	9	15	16	6	40	2	33	68
		1.000	13	18	1	34	30	12	48	17	40	66
S-143	polymerized sodium salts of alkylnaphthalene sulfonic acid [Darvan No. 1] (18)	0.001	4	12	−6	7	−1	6	35	15	13	34
		0.010	−1	10	0	23	3	−2	34	10	19	37
		0.100	2	8	−8	11	9	10	27	5	15	41
		1.000	−8	0	−1	3	7	10	27	11	3	31
S-144	polymerized sodium salts of substituted benzoid alkyl sulfonic acids [Darvan No. 2] (18)	0.001	3	5	1	6	7	−4	31	10	11	21
		0.010	4	5	−1	8	15	−8	33	12	4	27
		0.100	−1	8	4	2	8	1	29	−1	7	23
		1.000	−4	8	−9	2	−1	−2	23	4	1	25

CATIONIC SURFACTANTS												
S–010												
ethanolated alkyl guanidine-amine	0.001	8	4	9	13	6	0	34	28	16	43
complex [Aerosol C–61] (2)	0.010	2	8	1	9	–2	–3	32	37	16	49
	0.100	6	16	17	2	8	9	38	37	20	58
	1.000	6	32	9	14	12	18	58	49	17	67
S–016												
stearamidopropyldimethyl-β-hydroxyethylammonium dihydrogen phosphate [Cationic SP] (2)	0.010	2	5	1	8	5	2	32	34	30	36
	0.100	7	5	5	19	7	1	35	38	50	50
	1.000	8	9	2	4	6	13	42	41	22	61
S–019												
coco amine acetate, technical [Armac C] (4)	0.010	9	7	5	10	9	22	39	1	17	28
	0.100	–7	19	3	4	13	4	42	8	23	55
	1.000	27	53	31	50	40	51	78	80	82	51
S–021												
[mono-alkyl polyoxyethylene amine, alkyls from soybean amine] [Ethomeen S/15] (4)	0.001	1	5	–8	2	–1	–4	32	22	24	52
	0.010	–1	–2	4	8	21	7	36	24	30	61
	0.100	5	16	–2	22	19	2	44	39	25	65
	1.000	33	56	32	53	59	32	74	72	81	82
S–022												
[mono-alkyl polyoxyethylene amine, alkyls from tallow amine] [Ethomeen T/15] (4)	0.010	–1	9	6	6	13	8	24	–1	26	26
	0.100	6	17	4	18	14	1	24	12	37	46
	1.000	19	39	16	26	43	16	50	59	68	72
S–023												
coco trimethyl ammonium chloride, technical [Arquad C] (4)	0.010	7	9	–3	4	18	14	26	0	40	42
	0.100	13	33	3	–2	27	26	44	29	34	65
	1.000	29	55	37	55	53	57	88	76	90	78
S–026												
[mono-alkyl polyoxyethylene amine, alkyls from soybean amine] [Ethomeen S/25] (4)	0.001	15	18	18	12	10	–1	27	16	18	51
	0.010	10	16	16	19	16	–12	26	23	28	60
	0.100	7	43	17	25	34	2	35	34	44	66
	1.000	17	59	28	38	58	18	56	66	77	72
S–029												
alkylbenzyl quaternary ammonium halide [AHCO DD 50] (5)	0.010	7	0	1	10	8	6	25	30	21	36
	0.100	4	5	3	8	13	7	37	42	9	59
	1.000	54	90	63	80	88	51	78	71	66	84
S–121												
p–diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride [Hyamine–1622] (16)	0.010	1	2	3	8	2	–2	25	25	20	34
	0.100	1	16	6	9	16	10	41	47	12	67
	1.000	46	71	64	48	72	42	63	70	47	75
S–122												
alkyl (C_8–C_{18}) tolyl methyl trimethyl ammonium chlorides [Hyamine-2389] (16)	0.010	11	20	23	17	16	10	41	13	21	56
	0.100	4	19	5	6	20	14	44	4	14	78
	1.000	32	43	40	28	52	16	54	31	24	77

Surfactant number	Chemical description [Proprietary name] (Source)[a,b]	Conc. (% w/v)	Corn					Soybeans				
			Water	Dalapon 1 lb/A	2,4-D ¼ lb/A	DNBP ½ lb/A	Amitrol ¼ lb/A	Water	Dalapon 1 lb/A	2,4-D ¼ lb/A	DNBP ½ lb/A	Amitrol ¼ lb/A
AMPHOLYTIC SURFACTANTS												
S–071		0.001	6	5	7	6	6	2	33	40	32	52
		0.010	–4	3	–9	6	–3	1	36	32	35	51
	C-cetyl betaine [Product BCO] (11)	0.100	–4	11	15	10	1	0	34	38	30	58
		1.000	7	34	–3	10	11	12	54	42	46	71
S–075		0.001	3	4	14	10	–2	1	32	32	25	46
		0.010	6	4	12	10	5	2	34	38	22	56
	long chain amine [Alkanol EA] (11)	0.100	0	9	2	20	4	6	35	39	23	56
		1.000	–3	12	15	7	3	6	48	38	24	51
BLENDED SURFACTANTS												
S–058		0.001	7	6	7	23	19	8	32	10	24	24
	alkylarylpolyoxyethylene glycols;	0.010	0	13	12	11	16	7	30	18	15	38
	free fatty acids; isopropanol	0.100	11	14	13	23	20	4	47	14	21	51
	[Colloidal X–77] (9)	1.000	18	21	20	20	28	28	46	33	64	70
S–146		0.001	6	8	4	24	13	7	28	14	18	28
		0.010	2	14	13	10	10	6	35	14	11	42
	[Antarate 9181] (3)	0.100	0	13	10	6	12	0	34	22	33	41
		1.000	7	20	9	23	14	8	43	17	41	56
S–155												
	emulsifiable A–C polyethylene 629; fatty	0.010	0	16	0	12	6	–4	16	7	21	32
	acid-amine concentrate; alkyl aryl sul-	0.100	8	5	2	6	6	0	14	9	15	36
	fonate [Plyac Spreader-Sticker] (1)	1.000	5	4	8	19	12	8	27	9	42	44

[a]Sources of surfactants.

Number	Name and Address of Industrial Organization
1	Allied Chemical Corporation, General Chemical Division 40 Rector Street, New York 6, New York
2	American Cyanamid Company, New Products Development Dept. 30 Rockefeller Plaza, New York 20, New York
3	Antara Chemicals, Division of General Dyestuff Corp. 435 Hudson Street, New York 14, New York
4	Armour and Company, Chemical Division 1355 W. 31st Street, Chicago 9, Illinois
5	Arnold, Hoffman and Company, Inc., Technical Service Dept. 55 Canal Street, Providence 1, Rhode Island
6	The Atlantic Refining Company, Chemical Products Section 260 South Broad Street, Philadelphia, Pennsylvania
7	Atlas Powder Company, Industrial Chemicals Dept. Wilmington 99, Delaware
8	Colgate-Palmolive Company, Research & Development Dept. 105 Hudson Street, Jersey City 2, New Jersey
9	Colloidal Products Corporation 100 Gate 5 Road, Sausalito, California
10	Dewey and Almy Chemical Company, Organic Chemicals Division Cambridge 40, Massachusetts
11	E. I. du Pont de Nemours & Company, Organic Chemicals Dept. Dyes & Chemicals Division, Wilmington 98, Delaware
12	Emulsol Chemical Corporation, Agricultural Chemical Development 59 East Madison Street, Chicago 3, Illinois
13	Hercules Powder Company, Agricultural Chemicals Laboratory Wilmington 99, Delaware
14	Monsanto Chemical Company, Inorganic Chemicals Division 710 North Twelfth Street, St. Louis, Missouri
15	The Proctor & Gamble Distributing Company P. O. Box 599, Cincinnati 1, Ohio
16	Rohm & Haas Company, Agricultural and Sanitary Chemicals Dept. Washington Square, Philadelphia 5, Pennsylvania
17	Union Carbide Chemical Company, Division of Union Carbide Corp. 30 East Forty-Second Street, New York 17, New York
18	R. T. Vanderbilt Company, Inc., Specialties Dept. 230 Park Avenue, New York 17, New York
19	Wyandotte Chemicals Corporation, Market Development Dept. Wyandotte, Michigan

[b]Surfactants included in these investigations were randomly selected by classes from a larger inventory containing other surfactants for which necessary physical and chemical data were available to permit effective evaluation. The mention of commercial products and companies anywhere in this paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

logarithmic concentration of surfactant above the critical concentration. S–145, however, failed to enhance the activity of 2,4–D at $^1/_{16}$ lb/A on corn. With DNBP activity on corn, the threshold for initial surfactant enhancement occurred at the 0.1–percent concentration.

Throughout these experiments the standard surfactant S–145 showed essentially no inherent phytotoxicity. At the 0.1- and 1.0-percent concentrations on soybean activity indices of four and five, respectively, were barely significant at the 5-percent level of confidence. The response was apparent as a slight general growth inhibition and localized retardation of mesophyll development, which resulted in a pock-like appearance of mature primary leaves.

Comparative evaluations of other surfactants.

From three to eight new surfactants were selected for examination in each of 15 successive experiments. All results have been grouped and reported separately for the nonionic-, anionic-, cationic-, ampholytic-, and blended-surfactant classes (Table 1).

No attempt is made here to discuss each of the individual surfactants included in these investigations. Throughout the experiments certain characteristic types of behavior of the surfactants prevailed. In addition, several surfactant-induced responses which modified characteristic behavior were disclosed. Since the reader may wish to review the detailed tabular data for specific purposes, the various surfactant effects are discussed in only broad generalities.

The most frequently encountered type of surfactant action was enhancement of the herbicidal activity. For the most part, such enhancements followed the same general patterns exhibited by standard surfactant S–145 discussed previously. Characteristically, the breakpoint or threshold for surfactant enhancement was found within the range of concentrations from 0.01 to 0.1 percent and the degree of enhancement was greatest at the 1.0-percent concentration of the surfactant. The levels of enhancement obtained with individual surfactant concentrations were usually of the same order of magnitude as those obtained with equivalent concentrations of the standard surfactant. Occasionally, however, marked increases above the levels of the standard surfactant were noted. This fact is illustrated in Figure 4, in which the relative enhancements of DNBP activity on corn are shown for the standard surfactant S–145 and for S–034, the sodium salt of an alkyl aryl sulfonate type of anionic surfactant. Examination of the detailed tabular data for the latter surfactant in Table 1 reveals that S–034 had considerable inherent phytotoxicity at the 1.0-percent concentration. In the case of DNBP treatments the total activity resulting from the combination of 1.0 percent of S–034 with DNBP was much greater than the sum of the individual phytotoxicities of the mixture components. Instances of additive effects were found when this surfactant was combined with other herbicides. An important consideration would appear to be that an individual surfactant did not necessarily behave similarly in combination with all herbicides on the two species. In those instances

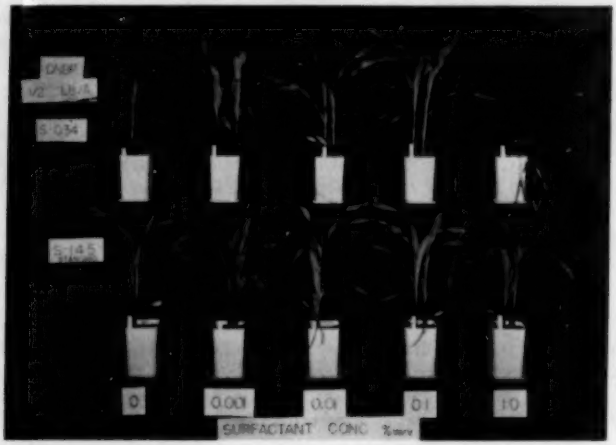

Figure 4. Surfactant enhancement of the activity of DNBP in corn.—S–034, a sodium salt of an alkyl aryl sulfonate, showed enhancement superior to that of S–145, the standard surfactant.

in which surfactant enhancement of herbicidal activity occurred, the enhancement appeared to be a function of the surfactant concentration but could be modified by inherent phytotoxicity of the surfactant in either a synergistic or additive manner.

In contrast to the enhancement of herbicidal activity just discussed, marked suppression of activity as a function of surfactant concentration was also observed. The materials which caused this type of action in the most striking manner were several polymerized salts of alkylaryl sulfonic acids, which are found in widespread industrial use as dispersing agents. Enhancement of activity, typically caused by S–145, and marked herbicide suppression, obtained with the anionic dispersant S–064, are contrasted in Figure 5. At the 0.1- and 1.0-percent concentrations of S–064 the characteristic expressions of 2,4–D activity in curvature, leaf malformation, and callus formation on the stem were progressively reduced. The activity indices for this series of treatments are given in Table 1.

Non-effective surfactant action, a third type of effect on herbicide activity, was found in every ionogenic class of surfactants. In such instances, the initial level of herbicidal activity remained unchanged over the entire range of surfactant concentrations used. The cationic surfactant S–010 (Table 1) was especially interesting, because it demonstrated all three of the basic types of surfactant activity. Typical surfactant enhancement was obtained with dalapon on both corn and soybean and with 2,4–D and amitrol on soybean. This surfactant suppressed the activity of DNBP on corn as concentration of the surfactant was increased from 0.001 to 0.1 percent. It also suppressed the activity of amitrol on corn at the two lower concentrations. However, it failed to affect the activity of DNBP on soybean.

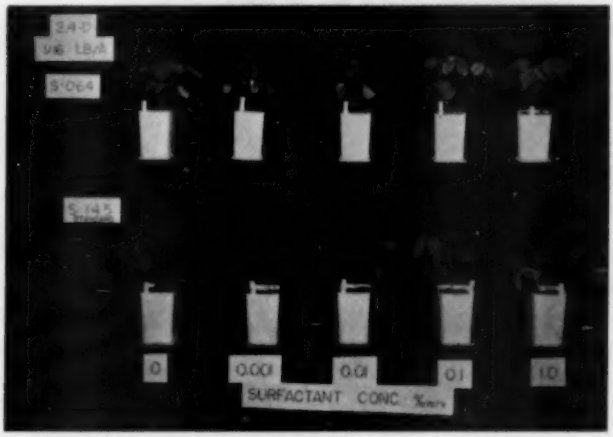

Figure 5. Comparative effects of two surfactants on the activity of 2,4–D in soybean.—S–064, a mono-calcium salt of polymerized arkyl aryl sulfonic acids, suppressed the activity of 2,4–D and S–145, the standard surfactant, enhanced the activity of 2,4–D as surfactant concentration was increased.

Many of the surfactants evaluated showed considerable herbicidal potential in the absence of herbicidal chemicals. Such phytotoxicities of surfactant solutions have been reported throughout the past 70 years (7, 13, 14, 15, 16). However, the magnitude of the phytotoxicities and the amount of surfactant required to elicit the various responses cannot be conveniently ascertained. In the present investigations, the application of a 1.0-percent concentration of a surfactant at a volume application rate of 40 gal/A deposited an amount of surfactant equivalent to 3.3 pounds of the active surfactant per acre. At the lowest concentration employed, 0.001 percent, the application rate amounted to 0.0033 pound of active surfactant per acre. The overall dosage effects of several closely related surfactants are graphically presented in Figure 6.

Three points, illustrated, in Figure 6, seem important. First, phytotoxicity, when it was found, was most characteristic of the 1.0-percent concentration of surfactant. Second, distinct selective phytotoxicities were exhibited by individual surfactants. Third, a number of surfactants exhibited stimulatory activity, particularly at lower concentrations. Such effects were noted throughout the course of these investigations and are indicated throughout Table 1 by negative values for various surfactant-water treatments.

Because of the manner in which the activity index is calculated for each treatment, negative values can arise only from two sources, namely, from growth increments in height or weight greater than those of the water-sprayed check treatments. The height and weight reduction percentages of soybean plants treated with several concentrations of S–113 in water illustrate the magnitude of growth

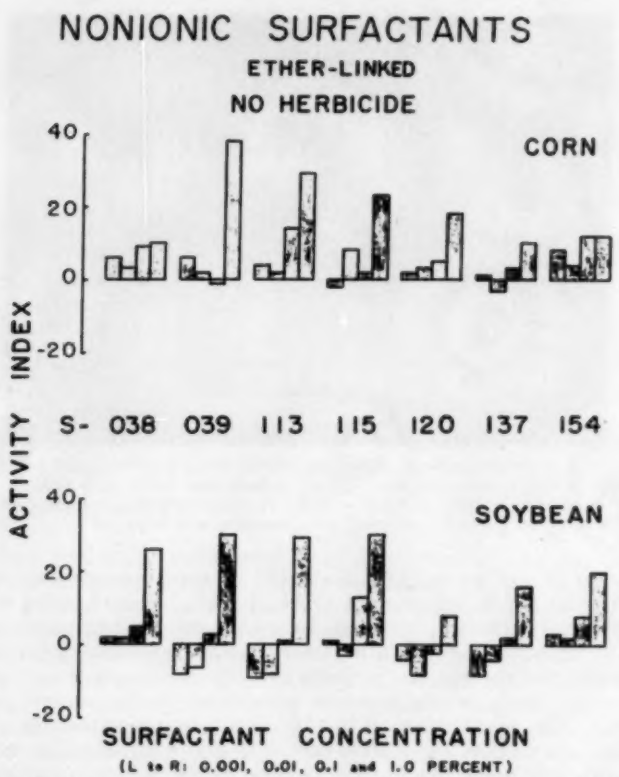

Figure 6. Biological activity of several closely related surfactants in corn and soybean.—The grouped bar graphs represent the activity indices of the four concentrations of each surfactant. S–numbers designate individual surfactants. Positive values indicate phytotoxicity; negative values, growth stimulatory activity.

stimulations (Table 2). Heights of plants treated with 0.001, 0.01, and 0.1 percent of S–113 were significantly greater than those of the controls. The range in heights from the 0.001- to 1.0-percent concentrations, however, fell just short of significance at the 5-percent level of confidence. The three lower concentrations of S–113 failed to give significant increases in the weights of soybean plants. Nevertheless, the general trend of weight changes from a 17-percent stimulation at 0.001 percent to a 31-percent reduction at 1.0 percent of S–113 represented a highly significant range difference. The activity indices to which these values contributed (Table 1 and Figure 6) reflected highly significant growth promotions at the 0.001- and 0.01-percent concentrations—values of −9 and−8, respectively—but failed to show any of the 24-percent height stimulation at the 0.1-percent

Table 2. Effects of concentration of surfactant S–113 on growth of soybean plants. —Negative values indicate percentage of growth stimulation; positive values, percentage of growth inhibition.

S–113 Percent w/v	Height reduction	Weight reduction
0.001	–25*	–17
0.01	–22*	–14
0.1	–24*	–8
1.0	–8	31**
LSD: P = 5%	17.9	19.6
P = 1%	27.1	29.7

*, ** Values represent differences at 5% and 1% levels of probability, respectively, from growth in water-sprayed check treatments.

concentration. Thus, many of the growth promotions actually observed in the course of the evaluation studies may be masked in the activity indices by positive values arising from visual ratings.

The structural complexities of surfactant molecules render the systematic naming of these compounds extremely difficult. For example, surfactants S–038 and S–154, data for both of which are included in Figure 6, were carried in the inventoried stock as an alkyl phenyl polyethylene glycol ether and as an alkyl phenoxy polyoxyethylene ethanol, respectively. Recent information revealed that these two proprietary materials contain the same surface-active ingredient, viz. the nonylphenyl ether of polyoxyethylene glycol. Although the experiments in which these two preparations were evaluated were separated by more than 3 months, the activities of the two "surfactants" on the two test species were strikingly similar. Examination of the detailed data on these preparations in Table 1 reveals also that the combination effects of the surfactant with the different herbicides were also similar on both species. Such results provide additional support for the adequacy of the experimental techniques and procedures.

In final analysis, a number of individual effects attributable to surfactant components of herbicidal spray systems were identified. Three types of surfactant actions on herbicidal activity—(1) enhancement, (2) suppression, and (3) lack of effect—were found. Surfactants in themselves may be either growth stimulatory or phytotoxic, and such properties may modify the character of the surfactant-herbicide response of a test species. Another point for consideration is that no single surfactant effect was found to be correlated with any single ionogenic class of surfactants. Insufficient structural data prevented reclassification of surfactants by any other scheme.

In field investigations at Beltsville in 1960, post-emergence applications of surfactant-herbicide combinations were made with a logarithmic field sprayer. The standard surfactant, S–145, was logarithmically applied simultaneously with a constant rate of application of each of the 4 herbicides used in the greenhouse studies. Amitrol was logarithmically applied with individual concentrations of S–145, a surfactant which enhanced activity, and S–144, an anionic dispersant which suppressed activity. Test species

used were corn, soybean, peanut, safflower, rape, ryegrass, pigweed, and ragweed. All of the major surfactant effects obtained in the greenhouse evaluation studies were confirmed under field conditions.

DISCUSSION

In the present work, the measurement of surfactant effectiveness was restricted to its ultimate expression in biological activity of the various herbicides employed. No attempt was made to correlate surfactant effectiveness with changes brought about in such phenomena as spreading, wetting, adherence, penetration, and chemical interaction with the herbicides. Such determinations should follow logically the preliminary evaluations.

Most of the critical studies conducted in the past attempted to correlate effectiveness of surfactants with surface activities of the solutions employed. Hence, considerable attention was given to such physical quantities as surface tension, interfacial tension, and adhesion, or in other words, to the free-energy characteristics of the interfaces. Most organic substances in solution will modify the free-energy relationships of the solvent; however, surfactants are distinguished by their ability to bring about these changes in an extreme fashion (26). In aqueous systems, most surfactants cause a very rapid drop in surface and interfacial tensions at extremely low concentrations. Within a narrow concentration range, 0.01 to 0.1 percent, the rapid fall in surface tension and interfacial tension begins to level off and little subsequent change is experienced with additional increases in concentration. The base of the steep fall in tension coincides very closely with what has been termed the critical micellar concentration for a surfactant. At this concentration initial aggregation of surfactant molecules into colloidal micelles occurs. At higher concentrations, most surfactants probably exist as strong colloidal systems.

The present work confirms previous findings (28) that the major influences of surfactants upon herbicidal activity are to be found in the range of concentrations beyond which the greatest changes in surface and interfacial tensions occur. In addition to such changes in the free-energy relationships at phase interfaces, however, surfactants also modify the characteristic drop in electrical potential of a two-phase system (26). When one phase is in a finely dispersed state, as in an emulsion or a dispersed solid system, cataphoresis or movement of charged particles under the influence of an electrical attraction may occur. The importance of this phenomenon to the movement of a pesticidal chemical from the spray solution into the plant requires detailed investigation. Other bulk properties of surfactant solutions should also receive much more attention. Marked changes in electrical properties, osmotic properties, diffusion properties, solubility and solubility-temperature relationships, viscosity, optical properties, and solubilization effects occur in the range of concentrations in which surfactants affect changes in herbicidal activity. Thus, surfactant effects on herbicidal activity probably correlate best with the colloidal state of the surfactant system. The

data in this paper should prove valuable in designing future critical experiments to correlate surfactant actions with physical and chemical phenomena.

Despite the lack of explanation for the various types of surfactant effects encountered in these studies, immediate application of the information could be made in a number of applied fields. Direct application of the data to problems in pesticide formulation is obvious. The basic results were substantiated by field experimentation. A number of possibilities by which herbicidal spray systems could be tailor-made for specific weed and weed-crop problems were suggested by these investigations.

Judicious selection of surfactants and concentrations might greatly increase the efficiency of herbicidal applications in controlling resistant weed species, lessen the possibility of damage to desirable plants, and decrease the cost of herbicidal application. Many of the surfactants at certain concentrations may increase the activity of a herbicide on one species but not on another, or they may bring about specific suppression of the herbicidal activity. Since an individual surfactant may also behave differently with different herbicides, appropriate selection of the surfactant and its concentration could conceivably either (a) increase the weed-killing potential of a herbicide in a resistant crop or (b) decrease the injury to a partially sensitive crop without affecting the weed-killing potential of a given herbicide dosage. In other situations addition of surfactant may permit a significant reduction of the herbicidal dosage without loss in effectiveness of the herbicide, or it may step-up the effectiveness of a chemical for the control of troublesome resistant weeds. Other possibilities may occur to the reader.

The results reported herein have only reemphasized the importance of this promising field of investigation in weed control technology. By systematic evaluation and indexing of a large number of surfactants, the areas in which more critical investigation is needed for aqueous foliage sprays become more apparent. Similar evaluations should be made of the oil-in-water and water-in-oil emulsifiable systems and of the complete oil systems. The spectra of species, chemicals, and chemical formulations should be expanded. Also, surfactant influences on herbicides applied to the soil as sprays, dusts, or granules should not be overlooked. The ultimate solution of many surfactant problems in chemical weed control technology undoubtedly will require coordinated research in a number of physical, chemical, and biological disciplines.

SUMMARY

1. An experimental technique for the evaluation of the effects of surfactants in aqueous spray systems of several herbicides was developed.

2. Sixty-three surfactants, representative of anionic, cationic, nonionic, ampholytic, and blended-surfactant classes, were evaluated in the greenhouse for effects on the herbicidal activity of water-soluble forms of dalapon, 2,4–D, DNBP, and amitrol. Two represen-

tative test species, corn and soybean, were employed. A polyoxyethylene sorbitan monolaurate, S–145, was included as a reference standard in all experiments.

3. Surfactants exhibited three characteristic types of action on herbicidal activity. Most commonly, progressive enhancement of herbicidal activity was obtained with logarithmic increases in surfactant concentrations. Some surfactants brought about progressive suppression of the herbicidal activity, and still others were ineffective in changing the level of activity attributable to the herbicide alone.

4. Many surfactants showed marked inherent phytotoxicity at higher concentrations, and some were growth stimulatory at low concentrations in the absence of herbicides.

5. Surfactant effects were not necessarily the same on both species, with all herbicides, or at all initial levels of herbicidal activity. No correlation was found between any one of the effects of a surfactant and the ionogenic class to which the surfactant belongs.

6. Principal results obtained in greenhouse experiments were confirmed under field conditions.

7. The possible applications of surfactants to future weed control research are discussed.

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Variation in Morphological Characteristics and Dalapon Susceptibility within the Species *Setaria lutescens* and *S. Faberii*[1]

P. W. Santelmann and J. A. Meade[2]

Annual grasses continue to be a problem. Some of the most prevalent annual grass weeds are members of the foxtail *(Setaria)* genus. Although these species can be controlled chemically, farmers have often had failures. One possible cause of these failures could be variability within the species.

The presence of weed "varieties" or selections within species has long been known. Hansen (3) in 1918 mentions varietal differences in the appearance of Canada thistle (*Cirsium arvense* (L.) Scop). Gleason (2), Willard (10), and others (1, 9) discuss two or more forms of Canada thistle. Kephart (7), and Fernald (1), list varieties of quackgrass (*Agropyron repens* (L.) Beauv). Gleason (2) states that quackgrass is "highly variable in color from green to glaucous, in pubescence of sheaths, blades and rachis, in shape of glumes, and in presence and length of awns."

Hubbard (5) reported many varieties of *Setaria viridis* (L.) Beauv. and *S. italica* (L.) Beauv. He called *S. viridis* an extremely variable species, and described two varieties—var. *ambigua* and var. *Wernmanni*. He found *S. italica* to be exceedingly variable, and describes in detail 3 subspecies, 5 varieties, 6 subvarieties, and 6 formae. He also lists 8 cultural names attached to different varieties. In addition, he (6) reported many varieties and forms of *S. barbata* (Lam.) Kunth. Gleason (2) lists three varieties of *S. viridis*, differing primarily in bristle characteristics and growth habit. Hitchcock (4) notes variability in *S. macrostachya* H.B.K., and Fernald (1) in *S. verticillata* (L.) Beauv.

These experiments were carried out in an attempt to determine the extent of variability of morphological characteristics and herbicide susceptibility within the species *S. lutescens* (Weigel) Hubb. (yellow foxtail) and *S. faberii* Herrm. (giant foxtail).

Materials and Methods

During the summer of 1958, head samples of both giant and yellow foxtail were collected at various locations in Maryland. Most of the samples were collected in the coastal plain area of eastern Maryland, but a few were collected in the Piedmont area of central Maryland. All Maryland samples were collected when the heads were fully mature and shattering. Dr. J. Morre of Purdue University supplied one giant foxtail seed sample, and Dr. R. Peters of the

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University of Connecticut supplied one yellow foxtail seed sample. Fifteen giant foxtail samples and 8 yellow foxtail samples were collected in Maryland. The greatest distance between the sources of any two samples was 100 miles. The seed was air-dried and stored at room temperature. During the winter of 1958–59, the giant foxtail seed was subjected to cold temperatures for 3 weeks in order to improve the germination (8).

Each selection was planted in the field in two 13-foot rows, 1 foot apart, on May 6, 1959 after treating the area with sodium methyldithiocarbamate to sterilize the soil. During the summer, notes were made on the growth characteristics of the plants. For each selection, at least 15 plants were individually observed and recorded, and the data averaged. While the plants were small, two-foot sections of each row were treated with two pounds of dalapon (sodium 2,2–dichloropropionate) per acre, and visual estimates of the degree of injury made at various dates.

In the fall of 1959, the selections of each species were seeded in greenhouse flats and treated at the two-leaf stage with two pounds of dalapon per acre and at the four-leaf stage with four pounds per acre.

RESULTS AND DISCUSSION

Considerable variation was found in the growth habit of the yellow foxtail collected from various locations. As indicated in Table 1,

Table 1. Variation in vegetative and reproductive growth between various selections of *Setaria lutescens* (Weigel) Hubb. grown in the field (each measurement is an average of 15 to 20 plants). 1959.

Origin, county or state	Plant height cm 8/6	Greenhouse leaf width mm	No. of tillers 8/6	Lodged 9/2	Heads per plant 8/6	Seed stage* 8/18	Pct. seed shattered	
							8/18	9/2
Wicomico	35	5	20.4	Yes	18.9	HD	70	85
Dorchester	43	2	21.8	No	17.2	SD	5	75
Caroline	42	2	26.9	Yes	26.1	HD	30	90
Caroline	40	–	15.3	Yes	12.3	SD	30	70
Talbot	25	3	13.1	No	7.6	SD	5	75
Queen Anne	32	–	14.8	No	8.0	M	0	70
Montgomery	53	5	20.1	No	19.7	HD	30	90
Prince George	41	4	14.5	No	12.6	HD	60	95
Connecticut	41	3	14.5	Yes	14.5	HD	50	85

*"M" indicates milk stage, "SD"—soft dough, "HD"—hard dough.

some were quite prostrate, branching out horizontally from the crown and reaching a maximum height of 25 cm. above the ground. Others consistently grew upright, reaching a maximum height of 53 cm. There was also considerable variation in leaf width (2 to 5 mm) and in amount of lodging. On September 2, four of the selections had lodged severely; the others remained upright. Tiller numbers varied from an average of 13.0 to 26.9 per plant. Three selections had severe stem rust injury; the rest did not. In general, there was little variation within the rows of each selection.

As the selections matured in the fall, there was considerable variation in date of heading, number of heads per plant, and rate of seed development. Heads of some varieties had emerged by July 6, others had only begun to emerge on July 23. The average number of heads per plant varied from 8.0 to 26.1. The average seed stage on August 18 varied between selections from milk to hard dough, and 60 per cent of the seed had shattered from the heads of one selection. Another selection had not begun shattering seed.

The end of each row was sprayed with two pounds of dalapon per acre when the plants were small, and then injury ratings made on a scale of 0 to 10, in which 0 equalled no injury, and 10 equalled complete kill. All but three yellow foxtail selections were rated as 4 on July 23 (Table 3); the other three were rated higher. On August 18 some selections had almost completely recovered from the treatment (rated 2 or 3) and were producing seed. Other selections were essentially dead, stunted, and brown, with only traces of green color showing (rated 7, 8, 9), and did not produce heads or viable seed. Greenhouse treatments showed the same variability in dalapon susceptibility.

As shown in Table 2, all 16 of the giant foxtail selections had an upright type of growth, the average height between selections varying from 104 to 150 cm. The variation between selections in average

Table 2. Variation in vegetative and reproductive growth between various selections of *Setaria Faberii* Herrm. grown in the field (each measurement is an average of at least 15 plants). 1959.

Origin, county or state	Plant height cm 8/6	No. of tillers 8/6	Lodged 9/2	Heads per plant 8/6	Seed stage[b] 8/18	Pct. seed shattered	
						8/18	9/2
Worcester	140	9.5	No	8.5	D	10	5
Somerset	107	11.6	Yes	.6	–	0	5
Wicomico	135	8.5	No	3.5	D	0	5
Wicomico	147	12.0	No	8.1	D	30	60
Wicomico	112	14.0	No	4.7	D	0	10
Caroline	117	9.4	No	2.7	M	5	30
Dorchester	125	15.1	Yes	7.6	HD	10	60
Dorchester	129	7.5	Yes	4.0	D	10	50
Talbot	129	11.2	Yes	7.3	SD	0	45
Caroline	117	12.6	Yes[a]	5.2	D	0	50
Queen Anne	127	10.5	Yes	3.6	D	20	75
Kent	150	7.1	Yes[a]	5.9	HD	30	75
Cecil	104	8.1	Yes	1.3	M	0	5
Montgomery	135	10.3	Yes	7.8	HD	15	75
Howard	127	8.7	Yes	2.7	SD	5	15
Illinois	129	14.2	Yes	9.5	HD	10	80

[a]Severely lodged.
[b]"M" indicates milk stage, "SD"—soft dough, "D"—dough, "HD"—hard dough.

number of tillers was from 7.1 to 15.1. As with the yellow foxtail, some selections lodged quite severely, some slightly, and some not at all. In vegetative growth, the giant foxtail selections were taller, had fewer tillers, and appeared to be less variable than the yellow foxtail selections.

Some plants of giant foxtail had begun to head on July 6, others did not begin heading until after July 28. The average number of

heads per plant varied from 0.6 to 9.5, and was not as closely correlated with tiller numbers as was the yellow foxtail. The average maturity varied widely between selections. On September 2, 75 per cent of the seed of some selections had shattered, but only 5 per cent of others.

Susceptibility to dalapon varied widely as shown in Table 3. On July 23, plant injury ratings varied between 2 and 6. About a month later some selections were rated 8 or 9, were practically dead, and no seeds were produced. Other selections were rated 3 or 4 and produced seed. In the greenhouse, all selections except one were fairly resistant to four pounds of dalapon per acre at the four-leaf stage. However, most were fairly susceptible to two pounds at the two-leaf stage.

Table 3. Variation in susceptibility of various selections of *Setaria lutescens* (Weigel) Hubb. and *S. Faberii* Herrm. to dalapon in the greenhouse and field. Data reported as injury rating scale, in which 0 equals no injury, 10 equals complete kill.

Origin, county or state	Field		Greenhouse		
	Setaria lutescens				
	7/25	8/18	11/23		12/8[b]
			A[a]	B[a]	A
Wicomico	4	3	7	2	10
Dorchester	4	6	5	2	5
Caroline	4	3	6	3	5
Caroline	4	2	–	–	–
Talbot	4	5	6	2	7
Queen Anne	4	7	–	–	–
Montgomery	8	4	7	3	10
Prince George	6	8	8	3	10
Connecticut	6	9	9	3	10
	Setaria Faberii				
	7/23	8/18	10/8		
Worcester	2	3	6	2	
Somerset	4	9	9	3	
Wicomico	2	8	7	3	
Wicomico	2	3	7	3	
Wicomico	4	5	8	3	
Caroline	4	4	8	2	
Dorchester	4	6	7	2	
Dorchester	4	7	7	2	
Talbot	4	5	9	2	
Caroline	6	8	9	3	
Queen Anne	4	5	7	2	
Kent	2	5	–	–	
Cecil	4	7	9	3	
Montgomery	2	4	8	6	
Howard	4	9	8	3	
Illinois	2	6	8	3	

[a]Stage A, 2 lb/A at the 2 leaf stage, plants 1½ inches tall. Stage B, 4 lb/A at the 4 leaf stage, plants 6 inches.
[b]On 12/8, all Stage B plants had recovered.

The variability between selections within a species can be important to weed research workers. In greenhouse work, for example, a worker may have seed from several selections of foxtail mixed together, such as from various small lots of seed collected in the field. This mixture of selections could lead to highly variable results.

Thus, it would be most desirable to use seed from only one lot for a given experiment. Also, the variability within species in dalapon susceptibility might explain some of the failures or partial failures in control of these species on the farm.

SUMMARY

During 1958, seed samples of 9 yellow foxtail *(Setaria lutescens* (L.) Beauv.) and 16 giant foxtail (*S. faberii* Herrm.) selections were collected from different locations. In 1959 these were seeded in individual plots in sterilized soil in the field. During the growing season the characteristics of each selection were recorded. The 25 selections were treated with dalapon in the field and in the greenhouse.

Wide variation was found between selections within each species in growth habit, height, leaf width, lodging, number of heads, rate of seed development, and dalapon susceptibility. These variations might be at least a partial cause of failures in the use of herbicides for foxtail control.

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Soil-incorporated Pre-planting Herbicides for Witchweed Control[1]

E. L. Robinson[2]

Witchweed [*Striga asiatica* (L.) Kuntze] is an annual parasite which was discovered in North Carolina and South Carolina of the southern United States in 1956 (1). This weed presents a hazard to the economic production of corn (2, 6), sorghum, sugarcane, and other host plants.

In field investigations, the sodium salt of 2,4–dichlorophenoxyacetic acid (2,4–D) and other formulations of this herbicide applied as post-emergence sprays to witchweed-infested sorghum have killed the newly attached and emerged witchweed plants without significant injury to the sorghum (4, 7). In laboratory studies, a 0.1 percent solution of 2,4–D applied to the surface of the soil killed the underground seedlings of witchweed to a depth of 6 inches (3).

In preliminary investigations at Whiteville, North Carolina, in 1957, pre-planting soil-incorporated treatments resulted in a more consistent kill of witchweed than either pre-or post-emergence treatments (5). The objective of the study reported herein was to evaluate several pre-planting soil-incorporated herbicides for the control of witchweed in corn.

Materials and Methods

In 1958 and 1959, field investigations were conducted on a relatively infertile, shallow phase, Lakeland sand at Whiteville, North Carolina. In both years, a 5–10–10 fertilizer at 600 pounds per acre was applied at planting time and 100 pounds per acre of additional nitrogen was broadcast when the corn was three feet high.

In 1958, the experiment was a randomized block with four replications. The plots were 12 feet wide and 40 feet long. The herbicides were applied to the surface of the soil and incorporated in the upper four inches by going over once with a double disk harrow. Corn hybrid Dixie–18 was planted seven days after the herbicides were incorporated. The herbicides used in 1958 were an alkanolamine salt of 2,4–D, the sodium salt of 2,3,6–trichlorobenzoic acid (2,3,6–TBA), 2–chloro–4,6–bis(ethylamino)–*s*–triazine (simazine), ethyl *N*,*N*–di–*n*–propylthiolcarbamate (EPTC), 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron), 3–(3,4–dichlorophenyl)–1,1–dimethylurea (diuron), 2–chloro–*N*,*N*–diallylacetamide (CDAA), 2–chloroallyl diethyldithiocarbamate (CDEC), and isopropyl *N*–(3–chlorophenyl) carbamate (CIPC).

The most promising herbicides as pre-planting soil-incorporated treatments in the 1958 investigations were the alkanolamine salt of

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2,4–D and the sodium salt of 2,3,6–TBA. In 1959, these two herbicides plus the sodium salt of 2,3,6–trichlorophenylacetic acid (fenac), 2,4–dichlorophenoxyacetamide, the monoethanolamine salts of 2–methoxy–2,3,6–trichlorobenzoic acid and 2–methoxy–3,6–dichlorobenzoic acid, and the sodium salts of mixed isomers of 1,2,3– and 1,2,4–trichlorobenzoic acid were further evaluated.

The design in 1959 was a randomized block with three replications. The soil type and fertilizer were the same as in 1958. Corn hybrid Dixie–18 was planted 5, 10, and 20 days following the pre-planting soil-incorporated treatments. The corn was cultivated twice during the growing season.

RESULTS AND DISCUSSION

The herbicides, their rates of application, and corn yields following the treatments in 1958 are presented in Table 1. The data in Table 1 show that of the treatments evaluated in 1958, only the alkanolamine salt of 2,4–D and the sodium salt of 2,3,6–TBA were effective in controlling witchweed as measured by corn yield. The other herbicides included in this study and listed in Table 1 in most

Table 1. Yields of corn following pre-planting, soil-incorporated applications of herbicides for witchweed control, 1958.

Herbicide	Rate per acre lb	Yield per acre[a] bu
2,4–D, alkanolamine salt	3	8.2
	6	3.6
	12	22.4
2,3,6–TBA, sodium salt	3	2.9
	6	16.9
	12	23.6
Simazine	4	0.6
	8	1.9
	12	0.6
EPTC	4	4.8
	8	0.0
	16	2.9
Monuron	1	1.6
	2	0.7
	4	1.8
Diuron	1	5.8
	2	1.0
	4	1.6
CDAA	4	2.2
	8	2.6
	16	4.0
CNEC	4	5.5
	8	5.0
	16	1.6
CIPC	4	0.9
	8	3.0
	16	1.9
Check	0	0.3

[a]Average of 4 replications except check, which is the average of 12 replications.

instances gave excellent control of ordinary weeds, but were not effective in controlling witchweed. The pest emerged and grew vigorously in all other treatments, and caused serious damage to corn, as indicated by the yields harvested.

The 1959 results, including the effect of the herbicides on corn stand and yields, are presented in Table 2. There were no significant differences in yields of corn due to time of planting after treatment in 1959, so the data for the plantings made 5, 10, and 20 days after the herbicide applications have been averaged in Table 2.

Table 2. Yields of corn following pre-planting, soil-incorporated applications of herbicides for witchweed control, 1959.

Herbicide	Rate per acre lb	Stand reduction pct	Yield per acre[a] bu
Fenac, sodium salt	4	10	69.2
	8	50	45.7
	12	65	12.0
2,3,6–TBA, sodium salt	4	0	47.3
	8	10	46.8
	12	25	49.9
2,4–D, alkanolamine salt	4	0	45.2
	8	0	25.5
	12	0	55.9
2,4–dichlorophenoxyacetamide	4	10	33.1
	8	60	41.4
2–methoxy–3,5,6–trichlorobenzoic acid, monoethanolamine salt	4	0	52.0
	8	10	39.1
	12	34	51.3
2–methoxy–3,6–dichlorobenzoic acid, monoethanolamine salt	4	0	20.3
	8	0	46.9
	12	0	35.8
Amiben, triethylamine salt	4	0	6.5
	8	0	30.6
	12	20	5.9
1,2,3– and 1,2,4–trichlorobenzoic acid	4	0	12.1
	8	0	12.6
	12	0	30.7
Check		0	19.4

[a]Average of 3 replications except check, which is average of 25 replications.

The alkanolamine salt of 2,4–D, the sodium salt of 2,3,6–TBA, monoethanolamine salt of 2–methoxy–3,6–dichlorobenzoic acid, and 2–methoxy–3,5,6–trichlorobenzoic acid were highly effective as pre-planting soil-incorporated treatments for the control of witchweed with little or no effect on corn stands.

The most promising herbicide in 1959 was the sodium salt of fenac. This herbicide at 4 pounds per acre resulted in the highest corn yield with little or no effect on corn stands. At 8 and 12 pounds per acre, fenac gave excellent control of witchweed but caused significant reductions in corn stands.

In order to determine the residual activity of the herbicides, the plots treated in 1959 were planted to corn, peanuts, soybeans, cotton,

and tobacco in 1960. The residual effects of fenac and 2,3,6–TBA applied in 1959 on several crops grown in 1960 are shown in Table 3. All crops, except corn, listed in Table 3 showed residual effects of the herbicides. In fenac treated plots the damage to soybeans and tobacco was severe. The sodium salts of TBA were also very injurious to tobacco. Further investigations are critically needed to understand more clearly the behavior and persistence of these compounds in soils.

Mixing the herbicide into the soil prior to planting a crop appears to offer several advantages for the control of witchweed as well as ordinary weed populations in corn and other crops. In the southern United States where widely different crops such as tobacco, cotton, peanuts, and vegetables, are produced, it is less hazardous to apply the herbicides as pre-planting soil-incorporated treatments before most of these crops are planted. The time of application for such treatments does not appear to be as critical as for pre- and post-emergence treatments. The greater consistency in weed control obtained with certain herbicides applied pre-planting is outstanding.

Table 3. The residual effects of pre-planting, soil-incorporated herbicides applied in 1959 for witchweed control in corn on the growth of several crops in 1960.

Herbicides applied in April 1959	Rate per acre	Crop injury in July 1960[a]				
		Corn	Peanuts	Cotton	Soybeans	Tobacco
Fenac, sodium salt.....	4	0	1	1	8	4
	8	0	3	3	9	8
	12	0	7	6	10	9
2,3,6–TBA, sodium salt...	4	0	1	1	1	3
	8	0	2	3	2	6
	12	0	5	5	4	7

[a]Crop injury rating = 0, no effect; 1, 2, 3—slight injury; 4, 5, 6—moderate injury; 7, 8, 9—severe injury; 10—all plants killed.

SUMMARY

The investigations with pre-planting soil-incorporated herbicides may be summarized as follows:

1. The alkanolamine salt of 2,4–D and 2,4–dichlorophenoxyacetamide at 8 to 12 pounds per acre were effective in controlling witchweed without significant reductions in stand of corn.

2. The substituted benzoic acids, especially the sodium salt of 2,3,6–TBA, monoethanolamine salt of 2–methoxy–3,6–dichlorobenzoic acid, and 2–methoxy–3,5,6–trichlorobenzoic acid, gave excellent control of witchweed without significant reduction in stands or injury to the crop.

3. The most promising herbicide was the sodium salt of fenac. This herbicide, at four pounds per acre, was highly effective in controlling witchweed without serious injury to the corn. At eight pounds per acre or more, fenac gave excellent control of witchweed but caused serious reductions in corn stands. These studies suggest

evaluating fenac at lower rates, using more effective incorporation, and timing techniques.

4. Preliminary investigations of the residual activity of the 1959 treatments on corn, peanuts, soybeans, cotton, and tobacco in 1959 indicated that some of the herbicides, especially fenac, remained in the soil at least 12 months in quantities sufficient to damage tobacco and soybeans. Further investigations are needed on the residual activity of these treatments on subsequent crops in the rotation.

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Effect of Herbicides on Salmonberry and on Sitka Spruce and Western Hemlock Seedlings[1]

JAMES T. KRYGIER and ROBERT H. RUTH[2]

IN coastal Oregon, mild climate, highly productive soil, and annual precipitation of as much as 130 inches combine to produce an exceptionally high rate of forest growth. When these forest stands are harvested, however, brush establishes rapidly in the openings, competes strongly with conifer reproduction, and eventually causes a reduction in timber yields. Such losses are serious in a region which depends largely on the timber resource.

Salmonberry *(Rubus spectabilis)* is responsible for a large part of the coastal brush problem. It is hard to kill, and research has necessarily been extensive on both foliage and basal treatments. This paper describes herbicide screening trials on salmonberry and seedlings of western hemlock *(Tsuga heterophylla)* and Sitka spruce *(Picea sitchensis)*, the objective being to control salmonberry with minimum damage to the tree seedlings. Supplemental data were taken on herbicide effectiveness as influenced by weather and season of application.

Salmonberry is distributed from Alaska to northern California and inland to northern Idaho and western Montana. It attains maximum size in stream bottoms in the coastal forest, where it is associated with Sitka spruce, western hemlock, red alder *(Alnus rubra)*, and Douglas fir *(Pseudotsuga menziesii)*. Associated brush species that also compete with conifer seedlings are western thimbleberry *(Rubus parviflorus)*, salal *(Gaultheria shallon)*, and vine maple *(Acer circinatum)*.

The ecology of salmonberry has not been thoroughly investigated, but some characteristics have been observed. The species propagates from suckers and from seed and is intermediate in shade tolerance. It often predominates on cutover or burned-over areas or in understocked areas of coastal forests. Soil disturbance and increased sunlight apparently stimulate germination of seed stored in the duff. Dense stands of seedling salmonberry will often occur on cutover land, even though the species was not prevalent under the forest canopy. When salmonberry is present in the understory, the tops, roots, and rhizomes are cut and broken during logging. Vigorous resprouting follows and competition is frequently more than natural or planted conifer seedlings can cope with (Figure 1). Broadcast burning of logging slash retards brush for about one season, but

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Figure 1. Salmonberry brush on this clear-cut area is 2 to 6 feet tall, typical of that sprayed in the chemical screening trials. The wood stake indicates a planted Sitka spruce seedling overtopped by brush.

subsequent sprouting is dense, and vigorous competition continues as an obstacle to the attainment of full stocking of conifer seedlings.[3]

EXPLORATORY STUDIES

In 1952 and 1953, 58 plots of 400 square feet each were established in typical salmonberry brush areas on four logging clearcuts. Herbicides were applied to the foliage in late spring and early summer, and the results were observed for the next two growing seasons.

Best results were obtained with propylene glycol butyl ether (PGBE) and butoxy ethanol esters of 2,4,5–trichlorophenoxyacetic acid (2,4,5–T) and mixtures of 2,4,5–T with 2–methyl–4–chlorophenoxyacetic acid (MCPA) and with 2,4–dichlorophenoxyacetic acid (2,4–D). Concentrations ranged from 1.75 to 18.8 lb/A in a water carrier. As high as 50 percent of salmonberry plants were killed on plots receiving high concentrations, but sprouting was general. Foliage applications of ammonium sulfamate (AMS) at 140 lb/A resulted in dense resprouting and little control. PGBE esters of 2,4–D + 2,4,5–T mixed with trichloroacetic acid (TCA) (1:1:2.4) in water at 3.75 + 3.75 + 9 lb/A resulted in poor control of salmonberry but good control of understory grass. Helicopter application of 2,4–D + 2,4,5–T in oil and in water; 2,4,5–T amines and esters with MCPA in water; and mixtures of 2,4–D + 2,4,5–T with TCA

[3]Ruth, Robert H. Plantation survival and growth in two brush-threat areas in coastal Oregon. Pacific Northwest Forest and Range Expt. Sta. Res. Paper 17. 1956.

in water—all at 3 to 12 lb/A resulted in partial defoliation and only moderate control of salmonberry.

Results of these exploratory studies indicated that general observations from plots were not precise enough for meaningful evaluation of results. More accurate observational and statistical controls were needed.

METHODS

From mid-June to mid-July in 1954 and 1955, herbicides were applied to individually tagged plants on plots established in a uniform stand of salmonberry brush 2 to 4 feet tall. Unlike many *Rubus* species, aerial portions of individual salmonberry plants are relatively well differentiated and not difficult to identify. However, there may have been rhizomatic connections between some plants. The plants had from 5 to about 25 stems per plant. There were 10 plants per plot, and three plots were established for each treatment. Spacing between plots was sufficient to prevent contamination from spray applications on nearby plots. Plot treatments were selected at random, and foliage was sprayed to the point of runoff with a fine, fan-shaped spray. Good coverage of the foliage required a spray volume of about 100 gal/A.

Screening trials in 1954 involved three herbicides: 2,4,5–T; 2–(2,4,5–trichlorophenoxy)propionic acid (silvex); and 2,4–D + 2,4,5–T. Concentration was 3 lb aehg in water.

Screening trials in 1955 involved six herbicides: 2,4–D; 2,4,5–T; 2–(2,4–dichlorophenoxy)propionic acid (2–(2,4–DP)); silvex; 2,3,6–trichlorobenzoic acid (2,3,6–TBA); and 3–amino–1,2,4–triazole (amitrole). Various formulations, concentrations, and combinations of these herbicides expanded the number of treatments to 36. The carrier was water plus 5% kerosene, except that no kerosene was used in amitrole and 2,3,6–TBA treatments.

In 1955, supplementary plots were established to explore the effects on salmonberry of June and September seasonal treatments and a technique of application during wet and dry weather. In the seasonal treatments, three formulations of 2,4,5–T were applied in June and to adjacent plots in September at 2- and 4-lb aehg concentrations each. In the study of technique of application, wet-weather treatments were those in which stems and lower as well as upper surfaces of leaves were sprayed with 2,4,5–T and with amitrole + 2,4–D + 2,4,5–T (1:1:1). Foliage was wet from rain or blowing coastal fog. These herbicides were also sprayed during dry weather on upper surfaces as was done in the major screening trials.

The treatments used in the 1954 and 1955 screening trials were applied the same years in a similar experimental design to assess tolerance of 3- to 5-year-old Sitka spruce and western hemlock seedlings.

Records made at the end of the first and second growing seasons for all treatments included height of parent salmonberry plants; number, height, and density of sprouts; and height of spruce and hemlock seedlings. Percent defoliation of salmonberry plants was

recorded for the first season after the 1954 treatments and for two seasons after the 1955 treatments. Analysis of variance was based on percent defoliation the second growing season for 1955 salmonberry trials. This provided a measure of total crown reduction, which included dead stems and recovery through refoliation and sprouting. Analysis of results on conifers was based on 2-year net change in height with dead seedlings rated as zero height.

RESULTS ON SALMONBERRY

Effects of herbicide, formulation, and concentration. In the 1954 screening trials, esters produced greatest first-year defoliation—94 to 100 percent on treated plants (Table 1).

Table 1. First-season defoliation and second-season sprouting of salmonberry treated in mid-July 1954, 3 lb aehg.

Herbicide and formulation	Per cent first-season defoliation	Second-season sprouting	
		Per cent plants with sprouts	Per cent sprout height of parent plant height
2,4,5–T			
Amine	48	93	83
PGBE esters	99	70	50
Silvex			
Amine	80	73	57
PGBE esters	100	60	47
2,4–D + 2,4,5–T PGBE esters	94	87	70

Several of the 1955 herbicide-formulation combinations resulted in almost complete residual defoliation 2 years after application (Table 2). These treatments, based on percent defoliation at the end of the second growing season, varied in effectiveness from almost 100 to about 10 percent defoliation. Analysis of variance showed that variation among treatment means was significant at the one percent level. Silvex ranked highest in defoliation with 2,4,5–T a close second. Amitrole was very effective but somewhat slower acting as indicated by less first-season defoliation. Mixtures of 2,4–D and 2,4,5–T produced a wide range of results but they generally provided unsatisfactory salmonberry control. When added to amitrole, 2,4–D + 2,4,5–T improved its first-year defoliation. A mixture of 2–(2,4–DP) + silvex ranked with herbicides producing the best results. Poorest and completely unsatisfactory results occurred with 2,4–D, 2–(2,4–DP), and 2,3,6–TBA.

Taken as a group the 4 lb aehg concentrations were significantly better than the 2 lb at the 5 percent level. There were several exceptions to this with amitrole and the PGBE esters of silvex both doing an effective job at 2 lb aehg. The treatment × concentration interaction, however, was not significant.

Effects of season, weather, and technique. June treatments with 2,4,5–T esters produced significantly better second-season defoliation

Table 2. Defoliation and resprouting following foliage treatments on salmonberry in June 1955.

Treatment		Per cent defoliation		Per cent plants totally defoliated and without sprouts second season[a]
Herbicide and concentration, lb aehg	Formulation	First season	Second season	
Silvex, 2	Butoxy ethanol ester	85	66	55
Silvex, 4	do	100	94	90
Silvex, 2	PGBE ester	99	98	85
Silvex, 4	do	99	99	90
2,4,5–T, 2	Butoxy ethanol ester[b]	92	75	42
2,4,5–T, 4	do	94	91	72
2,4,5–T, 2	PGBE ester	90	82	60
2,4,5–T, 4	do	100	97	95
2,4,5–T, 2	Solubilized acid	47	57	15
2,4,5–T, 4	do	98	95	80
2,4,5–T, 2	Solubilized polyglycol ester	91	68	40
2,4,5–T, 4	do	97	81	50
2,4–D + 2,4,5–T, 1 + 1	Butoxy ethanol ester	69	64	20
2,4–D + 2,4,5–T, 2 + 2	do	97	84	55
2,4–D + 2,4,5–T, 1.3 + 0.7	Butoxy ethanol ester[b]	64	59	22
2,4–D + 2,4,5–T, 2.7 + 1.3	do	80	58	22
2,4–D + 2,4,5–T, 1 + 1	Solubilized acid	29	27	5
2,4–D + 2,4,5–T, 2 + 2	do	76	68	35
Amitrole, 2	—	55	91	55
Amitrole, 4	—	64	94	75
Amitrole, + 2,4–D + 2,4,5–T, 2 + 1 + 1	PGBE ester	87	81	60
Amitrole, + 2,4–D + 2,4,5–T, 4 + 1 + 1	do	99	87	45
2–(2,4–DP) + silvex, 1 + 1	Butoxy ethanol ester	90	80	45
2–(2,4–DP) + silvex, 2 + 2	do	97	91	65
2–(2,4–DP), 2	PGBE ester	15	15	0
2–(2,4–DP), 4	do	65	39	20
2,4–D, 2	Solubilized polyglycol ester	12	21	0
2,4–D, 4	do	38	16	5
2,3,6–TBA, 2	Sodium salt	21	17	0
2,3,6–TBA, 4	do	24	3	0

[a]Plants not considered killed because many sprouted the third season.
[b]Average of 2 or 3 treatments with butoxy ethanol formulations containing slightly different components.

than September treatments (Table 3). Again it was found that 4 lb concentrations were significantly better for salmonberry control than 2 lb concentrations. There was no significant difference between the different ester formulations. The formulation × season interaction was significant.

Table 3. Second season defoliation of salmonberry treated with three formulations of 2,4,5–T in June and September 1955.

Ester formulation and concentration, lb aehg	Per cent second-season defoliation	
	June treatment	September treatment
PGBE, 2	82	42
PGBE, 4	97	69
Butoxy ethanol, 2	72	85
Butoxy ethanol, 4	93	78
Solubilized polyglycol, 2	68	56
Solubilized polyglycol, 4	81	87
Average	82.2	69.5

RESULTS ON SPRUCE AND HEMLOCK

Effects of herbicide and formulation. In the 1954 tests on spruce seedlings no mortality occurred, and differences in height growth between herbicide treatments (at 3 lb aehg) were not significant. In the 1954 tests on hemlock, mortality varied from 0 to 20 percent and differences in height growth between treatments were highly significant. The PGBE esters of silvex and 2,4,5–T caused less reduction in growth and less mortality than the amines (Table 4).

Table 4. Defoliation, mortality, and height growth of Sitka spruce and western hemlock seedlings, sprayed mid-July 1954, 3 lb aehg.

Herbicide and formulation	Spruce			Hemlock		
	Per cent first-season defoliation	Per cent second-season mortality	Av. 2-year net change in height, in.[a]	Per cent first-season defoliation	Per cent second-season mortality	Av. 2-year net change in height, in.[a]
2,4,5–T						
Amine	7	0	7.7	65	20	−2.3
PGBE esters	7	0	7.7	51	0	3.4
Silvex						
Amine	6	0	5.6	76	13	−1.4
PGBE esters	3	0	8.8	28	0	7.9
2,4–D + 2,4,5–T						
PGBE esters	15	0	5.8	68	7	−1.8
Check	0	0	10.3	0	0	8.8

[a]Net change in height was difference between the sum of original heights on 30 trees before spraying and the sum of their heights at the end of the second season after spraying. Dead seedlings were rated zero height.

In the 1955 tests spruce again displayed better resistance to the herbicides than hemlock, although some treatments caused severe reductions in growth and high mortality (Table 5). Hemlock proved significantly less resistant to the herbicides than spruce. For each species, net changes in height due to treatments were significant at the 1% level. For both species combined, 4 lb aehg concentrations did significantly more damage to the seedlings than 2 lb concentrations.

Effect of weather. Weather at time of treatment had little effect on subsequent height growth or mortality of seedlings. Spruce sustained less damage than hemlock for both the PGBE esters of 2,4,5–T and the mixture of amitrole, 2,4–D, and 2,4,5–T during both wet and dry weather.

SUMMARY

Screening trials on salmonberry on the Oregon coast indicated that several herbicide formulations applied as foliage sprays show promise for control of this species. Propylene glycol butyl ether (PGBE) esters of silvex and 2,4,5–T gave the best results followed closely by butoxy ethanol formulations of these same herbicides and by solubilized acid and solubilized polyglycol esters of 2,4,5–T. Amitrole also ranked with the most effective herbicides. It provided

less defoliation the first year but this was improved by addition of 2,4–D ± 2,4,5–T. Best selectivity was obtained with the PGBE esters of 2,4,5–T which were effective on salmonberry and did not seriously damage intermingled Sitka spruce and western hemlock seedlings. Amine formulations were ineffective on salmonberry and destructive to hemlock seedlings. Unsatisfactory results occurred from applications of 2,4–D, 2–(2,4–DP), and 2,3,6–TBA. Few salmonberry plants were killed by even the best treatments, but control was adequate to greatly improve establishment of tree seedlings.

Table 5. Two-year height growth and mortality of spruce and hemlock seedlings, sprayed June 1955.

Treatment		Av. 2-year net change in height in[a]		Per cent mortality	
Herbicide and concentration, lb ae/ag	Formulation	Spruce	Hemlock	Spruce	Hemlock
Silvex, 2	Butoxy ethanol ester	8	−6	8	32
Silvex, 4	do	−5	−18	26	87
Silvex, 2	PGBE ester	8	2	0	21
Silvex, 4	do	−2	4	35	32
2,4,5–T, 2	Butoxy ethanol ester[b]	7	1	12	19
2,4,5–T, 4	do	−6	−7	46	40
2,4,5–T, 2	PGBE ester	6	8	0	4
2,4,5–T, 4	do	9	9	0	0
2,4,5–T, 2	Solubilized acid	5	4	0	10
2,4,5–T, 4	do	0	−13	30	89
2,4,5–T, 2	Solubilized polyglycol ester	10	7	0	0
2,4,5–T, 4	do	3	4	17	0
2,4–D + 2,4,5–T, 1 + 1	Butoxy ethanol ester	0	−4	16	41
2,4–D + 2,4,5–T, 2 + 2	do	−6	−14	61	85
2,4–D + 2,4,5–T, 1.3 + 0.7	Butoxy ethanol ester[b]	0	−3	33	36
2,4–D + 2,4,5–T, 2.7 + 1.3	do	−2	−6	38	28
2,4–D + 2,4,5–T, 1 + 1	Solubilized acid	7	−1	3	33
2,4–D + 2,4,5–T, 2 + 2	do	5	1	4	7
Amitrole, 2	—	11	4	0	28
Amitrole, 4	—	4	−3	18	32
Amitrole + 2,4–D + 2,4,5–T, 2 + 1 + 1	PGBE ester	8	6	0	3
Amitrole + 2,4–D + 2,4,5–T, 4 + 1 + 1	do	9	−1	4	11
2–(2,4–DP) + silvex, 1 + 1	Butoxy ethanol ester	−6	−14	58	88
2–(2,4–DP) + silvex, 2 + 2	do	−11	−11	89	100
2–(2,4–DP), 2	PGBE ester	13	11	0	4
2–(2,4–DP), 4	do	9	10	0	0
2,4–D, 2	Solubilized polyglycol ester	12	10	0	3
2,4–D, 4	do	−6	2	53	4
2,3,6–TBA, 2	Sodium salt	−1	0	30	14
2,3,6–TBA, 4	do	−10	−5	59	23
Check	—	10	8	0	0

[a]Net change in height was difference between the sum of original heights on 30 trees before spraying and the sum of their heights at the end of the second season after spraying. Dead seedlings were rated as zero height.

[b]Average of 2 or 3 treatments with butoxy ethanol formulations containing slightly different components.

Control of Field Bindweed and Other Perennial Weeds with Benzoic and Phenylacetic Acids[1]

A. F. Wiese and H. E. Rea[2]

The growth promoting activity of substituted benzoic acids and 2,4–dichlorophenoxyacetic acid (2,4–D) was reported in 1942 by Zimmerman and Hitchcock (17). Interest in benzoic acids as herbicides was revived in 1950 when Bentley (1) reported outstanding growth regulation with 2,3,6–trichlorobenzoic acid. Shortly thereafter several workers confirmed these results (4, 5, 18). Chlorinated benzoic acids have proven to be effective for eradication of field bindweed (6, 8, 11, 12), and many other perennial weeds.[3] At the present time these herbicides are labeled and sold for the eradication of field bindweed, leafy spurge, bur ragweed, Canada thistle, and Russian knapweed.

The period of soil residual effects from chlorinated benzoic acid appears to be related to the amount of rainfall or irrigation after the application of the herbicide (7, 13). Under dryland conditions the herbicides have been known to persist for 2 years in amounts toxic to wheat and sorghum but under irrigated conditions a normal crop of wheat was produced 6 months after application.

In 1935 phenylacetic acid, one of the first growth promoting chemicals discovered, was reported to induce responses in plants when it was applied either to leaves or soil in which plants were growing (2, 15). The potassium salt of phenylacetic acid was observed to be a growth regulator in 1937 (16). Later, workers showed that alpha–methoxyphenylacetic acid applied to plant leaves would move down the plant and into the soil or water surrounding the root system (3, 9). In 1958 Pybus, Wain, and Wightman (10) reported that 2,3,6–trichlorophenylacetic acid and 2,6–dichlorophenylacetic acid exhibited as much growth promoting activity as either 2,3,6–trichlorobenzoic acid or 2,4–dichlorophenoxyacetic acid. The experimental use of 2,3,6–trichlorophenylacetic acid for eradication of perennial weeds was reported in 1958 (14).

The purpose of this paper is to summarize several years research in which substituted benzoic and phenylacetic acids were compared with other soil sterilants for the eradication of several perennial weeds.

Methods and Materials

Applications of various formulations of 2,3,6–trichlorophenylacetic acid (fenac), 2,3,6–trichlorobenzoic acid (2,3,6–TBA), and

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[3]N. Central Weed Control Conf. and Western Weed Control Conf., Res. Reports for 1955, 1956, 1957, and 1958.

several polychlorobenzoic acid materials were made to field bindweed *(Convolvulus arvensis)*, woolyleaf franseria (bur ragweed) *(Franseria tomentosa)*, hogpotato (Indian rushpea) *(Hoffmanseggia densiflora)*, alkali sida *(Sida hederacea)*, Texas blueweed *(Helianthus ciliaris)*, and silverleaf nightshade *(Solanum eleagnifolium)* during 1957, 1958, and 1959.

The 2,3,6–TBA used contained 60% 2,3,6–TBA and 40% other isomers of trichlorobenzoic acid. Polychlorobenzoic acid materials used will be designated as PBA(80%), PBA(30%), and PBA(2%). PBA(80%) contained 81% trichlorobenzoic acids, 12% tetrachlorobenzoic acids, 5% pentachlorobenzoic acid and 2% dichlorobenzoic acids; PBA(30%) contained 30% trichlorobenzoic acids, 54% tetrachlorobenzoic acids, 11% dichlorobenzoic acids, and 5% pentachlorobenzoic acid; PBA(2%) contained 2% trichlorobenzoic acids, 74% tetrachlorobenzoic acids and 24% pentachlorobenzoic acid. PBA(80%), PBA(30%) and PBA(2%) contained approximately 19%, 8%, and less than 2%, respectively, of 2,3,6–trichlorobenzoic acid. Rates of application for the benzoic acid herbicides are expressed in terms of total acid content. Formulations of fenac tested were an amide, an amine salt, sodium salt, butylcellosolve ester and the acid formulated as emulsifiable oil. 2,3,6–TBA was formulated as a dimethylamine salt and as the acid prepared as 25% granules. PBA(30%) was formulated as a dimethylamine salt and a butylcellosolve ester. PBA(80%) and PBA(2%) were formulated as dimethylamine salts. The above chlorinated benzoic and phenylacetic acid herbicides were compared to sodium chlorate, anhydrous borax (61.5% B_2O_3), 3–(*p*–chlorophenyl)–1,1–dimethylurea (monuron), and 3–phenyl–1,1–dimethylurea (fenuron).

The experimental designs of the studies conducted were either randomized blocks or split plots of 1 or 2 square rods with 1, 2, or 3 replications. Weed control was evaluated by comparing the number of plants in one square meter area in the center of each plot from treated and untreated areas. Data reported in this paper were obtained 12 to 18 months after application of the herbicides. The experiments were conducted at various locations in the Panhandle of Texas under both dryland and irrigated conditions. All experiments were on Pullman silty clay loam soil, and the average rainfall in the area is 21 inches. Rainfall for 1957, 1958, 1959, and 1960 was 21, 23, 23, and 37 inches, respectively.

RESULTS AND DISCUSSION

Much of the data presented will summarize more than one experiment. The year or years of application and the total number of replications will be shown in each table.

Field bindweed.

There was very little difference in the toxicity of TBA and fenac to field bindweed when applied at 10 and 20 pounds per acre (Table 1). However, at 5 pounds per acre, fenac was much more toxic than TBA. The data also showed that 2,3,6–TBA was approximately twice

as toxic as PBA(30%). Ten and 20 pounds of 2,3,6–TBA gave 88% and 94% control while 20 and 40 pounds of PBA(30%) gave 87% and 92% control. 2,3,6–TBA was only slightly more effective than PBA(80%). Fenac and TBA applied at 20 and PBA(30%) applied at 40 pounds per acre were equal to, or slightly more effective than, sodium chlorate applied at 800 pounds per acre.

Table 1. Percent bindweed control obtained from fenac and various chlorinated benzoic acid herbicides, 1957, 1958.

Herbicide	Rate lb/A				Number of replications
	5	10	20	40	
Fenac	79	88	97		8
TBA	52	84	95		
TBA		88	94	98	17
PBA(30%)		69	87	92	
Sodium chlorate at 800 lb/A = 88					
TBA		87	93	97	15
PBA(80%)		71	93	94	
Sodium chlorate at 800 lb/A = 93					

The toxicity of the various chloro-substituted benzoic acids to bindweed appeared to be somewhat related to the amount of trichlorobenzoic acid in the particular material (Table 2). The average control obtained from 5, 10, and 20 pounds per acre of 2,3,6–TBA, PBA(80%), PBA(30%), and PBA(2%) formulated as amine salts was 76%, 73%, 67% and 59%, respectively. However, in view of the fact that PBA(2%) was effective in killing bindweed, toxicity cannot be entirely attributed to the trichlorobenzoic acid content of the herbicides.

Table 2. Average percent bindweed control from herbicides containing various amounts of trichlorobenzoic acid. Six replications, 1958.

Herbicide	Rate lb/A			
	5	10	20	Av.
2,3,6–TBA, dimethylamine salts	52	81	94	76
PBA(80%) dimemethylamine salts	53	78	87	73
PBA(30%) dimethylamine salts	40	70	91	67
PBA(2%) dimethylamine salts	33	60	83	59
PBA(30%), butylcellosolve ester	37	73	94	68
Sodium chlorate, 800 lb/A				87

In previously published studies (14), the amide formulation of fenac was not as toxic to bindweed as an amine formulation. Additional studies indicate that the toxicity of fenac to bindweed is not greatly affected when it is formulated as an amine salt, a sodium salt, an acid in an emulsifiable oil or as a butylcellosolve ester (Table 3). 2,3,6–TBA formulated as a dimethylamine salt or as an acid impregnated on granules were similar in toxicity. The formulating of

PBA(30%) as a dimethylamine salt or as a butylcellosolve ester did not affect the toxicity of this herbicide (Table 2).

The toxicity symptoms of chlorinated benzoic and phenylacetic acids on bindweed regrowth are very similar. Injured plants are stunted and have light colored, deformed leaves. When liquid formulations of 2,3,6–TBA are applied to bindweed, death of the top growth usually occurs in about 1 week unless rain falls shortly after application. Top growth kill is then delayed until the roots are killed by the herbicide. PBA(30%) usually does not give such rapid top kill. Fenac has very little toxicity to the top growth of field bindweed; consequently, death of the foliage usually does not take place until enough rainfall occurs to leach the material into the plant root zone.

Table 3. Percent bindweed control after treatment with various formulations of fenac and TBA. Two replications, 1958.

Herbicide	Rate lb/A				
	2.5	5	10	20	40
Fenac, sodium salt	61	84	85	100	—
Fenac, amine salt	59	90	91	99	—
Fenac, butylcellosolve ester	57	71	76	99	—
Fenac, acid, inemulsifiable oil	56	74	90	100	—
2,3,6–TBA, dimethylamine salt	—	51	93	97	100
2,3,6–TBA, acid on granules	—	—	82	99	100

Fenac appears to have a longer period of soil sterility than the substituted benzoic acids. Applications of fenac made in 1957 on dryland have prevented all growth of annual weeds through 1960. Applications of substituted benzoic acids made at the same time allowed some growth of summer annual grasses in 1959 and 1960. When the herbicides were applied in April, 1959, the soil preirrigated in May, planted to grain sorghum in June, and irrigated in the summer, yields in the fall of 1959 were 5500, 0, 2330, and 5440 pounds per acre, respectively, from untreated plots, and treatments of 20 pounds of fenac, 20 pounds of 2,3,6–TBA, and 40 pounds of PBA(30%) per acre. One year later, in 1960, yields of sorghum grain from the same plots were 4350, 3540, 5000, and 5240 pounds per acre, respectively.

Other perennial weeds.

Studies conducted during 1957, 1958, and 1959 indicate that fenac, 2,3,6–TBA, or PBA(30%) at 20, 20, and 40 pounds per acre, respectively, were generally as effective or more effective on woolyleaf franseria, hogpotato, alkali sida, and Texas blueweed than sodium chlorate, anhydrous borax, monuron, or fenuron at 800, 2560, 60, and 60 pounds per acre, respectively (Table 4). Fenac at 20 pounds per acre gave a minimum of 95 percent control for these weeds. 2,3,6–TBA at 20 pounds per acre gave a minimum of 91 percent control for all weeds except alkali sida, and PBA(30%) at 40 pounds per acre gave less than 90 percent control of alkali sida and Texas bluweed. Silverleaf nightshade was very difficult to eradicate and all

Table 4. Percent control of various perennial weeds obtained from applications of various rates of fenac, 2,3,6–TBA, PBA(30%), and other soil sterilizing herbicides.

Herbicide	Lb/A	Woolyleaf[a] franseria	Hog-potato[b]	Alkali[c] sida	Texas[d] blueweed	Silverleaf[e] nightshade
Fenac	5	72	100	20	60	40
	10	83	100	80	90	50
	20	95	100	100	98	57
	30	—	—	—	100	—
	40	—	—	—	—	93
	80	—	—	—	—	100
2,3,6–TBA	10	83	97	40	83	—
	20	91	100	80	96	—
	30	—	—	—	100	—
	40	100	100	95	—	—
PBA(30%)	20	76	96	30	80	62
	40	94	99	80	82	67
	60	—	—	—	80	69
	80	100	100	100	—	—
Sodium chlorate	800	96	95	90	65	17
Anhydrous borax	2560	54	97	10	96	77
Monuron	60	71	80	60	96	66
Fenuron	60	79	76	60	100	88

[a]Two studies conducted in 1958; one dryland in the fall and the other irrigated in the spring making a total of 4 replications.
[b]Studies conducted on dryland in falls of 1957 and 1958 with a total of 4 replications.
[c]Study conducted on dryland in fall of 1958 with 1 replication.
[d]Study conducted on dryland in fall of 1959 with 2 replications.
[e]Study conducted on dryland in the spring of 1958 with 2 replications.

rates of all herbicides tested except fenac at 40 and 80 pounds per acre gave less than 90 percent control. Fenuron at 60 pounds per acre gave 88 percent control. These results indicate that soil sterilization for control of silverleaf nightshade is not feasible because of the poor results obtained with rates of herbicides usually required for eradication of perennial weeds.

SUMMARY AND CONCLUSIONS

These tests indicate that fenac, 2,3,6–TBA, and PBA(30%) at 20, 20, and 40 pounds per acre, respectively, are either as effective or more effective for eradicating field bindweed, woolyleaf franseria, hogpotato, and alkali sida than 800 lb/A sodium chlorate, 2560 lb/A anhydrous borax, 60 lb/A monuron, and 60 lb/A fenuron. Silverleaf nightshade was not successfully eradicated with any of the above rates of soil sterilants. Fenac is more toxic to bindweed than 2,3,6–TBA at low rates of application. At equal rates of application, chlorinated benzoic acid herbicides containing a high percentage of trichlorobenzoic acid were most toxic to bindweed. 2,3,6–TBA and PBA(30%) were equally effective when PBA(30%) was applied at twice the rate of 2,3,6–TBA. However, activity was not confined to the trichloro substitutions, as PBA(2%), which contains mostly tetrachlorobenzoic acids, was also toxic to bindweed. Formulation did not greatly affect the toxicity of chlorinated benzoic acids. The

amide formulation of fenac was not as toxic to bindweed as other formulations.

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Breaking Dormancy in Seeds of *Cuscuta approximata*[1]

D. C. Tingey and Keith R. Allred[2]

Dodder lowers the yield and quality of alfalfa seed, interferes with harvesting, and adds to the cost of cleaning and processing the seed for market. There are at least three species of dodder that are troublesome in alfalfa seed fields in Utah: Large seeded alfalfa dodder *(Cuscuta indecora* Choisy var. neuropetala (Engelm.) Hitch.), Western field dodder *(Cuscuta campestris* Yuncker) and small-seeded alfalfa dodder *(Cuscuta approximata* Bab. var. urceolata (Kuntze) Yuncker)[3] (Figure 1).

Literature Review

Several investigators at the Utah Agricultural Experiment Station have worked on various phases of the dodder problem, but none reported data regarding germination of seeds of *C. approximata* (4, 5, 7, 8, 9).

Dormancy in seeds of many species of dodder seems to be due to impermeable seed coats. This condition is readily corrected by acid treatment or by scarification (2).

Gaertner (2) germinated seeds of different species of dodder obtained from herbarium specimens to determine how long they retained their viability. The only treatment given the seed was an immersion in sulfuric acid. Two small seeded species of dodder, *Cuscuta epilinum* (flax dodder) and *Cuscuta epithymum* (clover dodder), failed to germinate following the acid treatment. This was attributed to the loss of viability of these old seeds.

Gaertner (2) reported that Kinzel (3) obtained a moderate percentage germination of seeds of *C. epilinum* with the acid treatment. However, he was not able to get seeds of *C. europea* to germinate following the acid treatment, and concluded that dormancy might be due to some factor other than an impervious seed coat. Gaertner also reported that Mirande (6) had no difficulty in germinating dodder seeds and that seeds of small seeded species *(C. europea* and *C. epithymum)* germinated after one week at temperatures of 10° C to 12° C (50° F to 53.6° F).

Gaertner (2) stored seeds at room temperature and in a cold room at 1–3° C both dry and in moist sand and peat moss without any appreciable effect on the germination of seeds. She concluded as did Midgley (5) that alternate freezing and thawing did not improve the germination of seeds of *Cuscuta* spp. Gaertner used *C. Gronovii* and Midgley *C. indecora*. Dormancy in seeds of these species is due only

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[3]This species was designated *C. planiflora* (1); however, from specimens submitted to T. G. Yuncker, it was classified *C. approximata* (10).

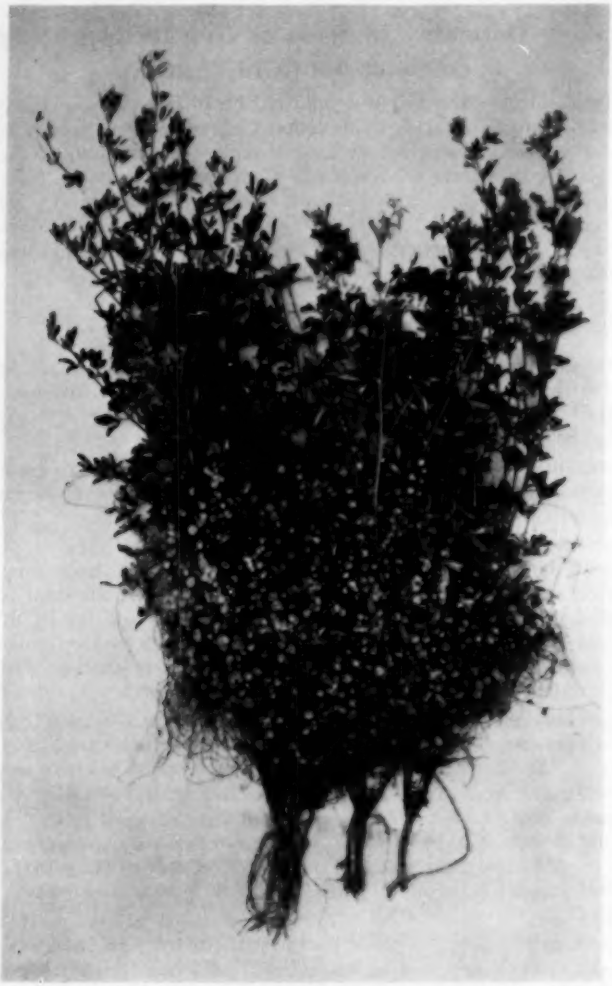

Figure 1. Alfalfa infected with *C. approximata* in bloom. Note the characteristic tendency for this species of dodder to remain restricted to the lower part of the plant.

to an impervious seed coat and is readily corrected by the acid or scarification treatments.

METHODS AND MATERIALS

Dodder seeds used in the study were obtained from various alfalfa seed producing areas in the state. Bulk lots were obtained from

alfalfa seed cleaning plants in Millard County. Some small lots were collected from individual alfalfa plants within seed fields. Seeds were germinated either on moistened blotting paper in covered petri dishes, or on blotting paper on moistened sand. Except where different temperatures were used as treatments, the seed was germinated in a seed germinator at a temperature of 72° F.

Number of days the seed was germinated varied with different experiments, but none was held for less than 10 days. Dodder seeds treated to break dormancy will germinate in a week or less under favorable conditions.

Duplicate samples of 100 seeds each were run. Except where indicated, the seeds received an acid treatment, followed by washing in running water.

The acid treatment consists of placing the seed in a glass beaker and adding about twice the volume of concentrated sulfuric acid and stirring thoroughly until the seed is well covered with the acid. The beaker is then placed in cold running water and the seed stirred frequently to prevent heating.

The scarifier used was one made by Fred Forsberg and Sons, River Falls, Minnesota. The optimum time for scarification of large seeded dodder in this machine for a 50-gram sample is about 5 minutes.

Seeds germinated at lower than room temperature were placed in refrigerators or refrigerated rooms. Seeds germinated at higher than room temperature were placed in constant temperature rooms and in the greenhouse. Except for the greenhouse, the temperatures would not vary more than about two degrees. There was no replication of temperatures. There were, however, replications within temperatures. Water was added as needed to keep the seed moist during germination, except for treatments where the seed was kept dry.

RESULTS AND DISCUSSION

Sulfuric acid or scarification breaks seed dormancy in C. indecora, but not in C. approximata. Data from one of a number of experiments for the purpose of breaking seed dormancy in dodder appear in Table 1.

Both acid and scarification were effective in breaking seed dormancy of *C. indecora,*[4] but not of *C. approximata.* Either acid or scarification apparently destroys the impervious seed coat of *C. approximata.* The treated seeds absorbed water and germination appeared to proceed as the tips of the thread-like embryo became visible and then growth ceased.

A small percentage of the seeds of *C. indecora* germinated without any treatment.

Scarification was not as effective as the acid treatment, probably because it had not been thorough enough. Optimum scarification is about as effective as the acid treatment in breaking seed dormancy in *C. indecora* and other species. Optimum time to operate the

[4]Dormancy in several other species of dodder found in Utah, including *C. campestris,* is readily broken by the acid or scarification treatment.

Table 1. Germination of dodder seed in 16 days following various treatments, 1956.

Treatment	Percent germination		
	C. indecora		*C. approximata*
	1955 seed	1956 seed	1956 seed
Control	12	8	0
Scarified	21	33	1
H_2SO_4 30 min.	58	63	1
60 min.	84	89	1
90 min.	77	72	1
150 min.	—	—	6

scarifier varies with seed lot, and even more with the amount of seed used in the machine.

Effect of temperature on germination of seeds of C. approximata. Acid treated seeds of *C. approximata* were placed to germinate at various temperatures ranging from 32° F to 98° F for a 40-day period.

Seeds of *C. approximata* gave low germination at all temperatures (Table 2). In two cases, 13 and 17 per cent of the seeds sprouted. These appeared to be chance variations more than any real treatment effect.

Seeds germinated in petri dishes at temperatures of 32° F, 37° F, and 45° F as listed in Table 2 were moved, after one month, to a germinator at room temperatures for another month.

Table 2. Germination of acid-treated seed of *C. approximata* in 40 days at various temperatures, 1957.[a]

Temperature degrees F.	Percent germination			
	1956 seed		1957 seed	
	Petri dish	Blotting paper on wet sand	Petri dish	Blotting paper on wet sand
32	0	0	0	0
37	0	0	0	0
40	0	0	13	0
44.6	0	0	7	2
45	0	0	0	6
50	1	7	17	4
68	0	0	0	0
70	2	3	0	3
80	0	0	0	0
98	0	0	0	0

[a]Seed in petri dishes at 32°, 37°, and 45°F. placed in germinator at 72°F. after one month (Table 3).

About a third of the 1957 seed, that had been wet chilled for a month, germinated after being placed at room temperature (Table 3). The reason for the difference in germination in the two lots of seeds is not known.

Additional experiments were conducted to further explore the effect of wet chilling on breaking dormancy in seeds of *C. approxi-*

mata. These involved a determination of the effect of wet versus dry chilling, and the interrelationship of the length of time the seed was treated with acid, the temperature during chilling, and the length of the chill period.

However, in the meantime it was thought that if wet chilling was responsible for breaking seed dormancy in *C. approximata*, it would be of interest to try germinating different seed lots of this species freshly collected from the field.

Table 3. Germination of acid-treated *C. approximata* seeds in 30 days at 72° F. following 30 days of wet chilling at various temperatures, 1957.

Temperature the first month degrees F.	Percent germination	
	1956 seed	1957 seed
32	1	27
37	6	38
45	5	31

Two lots of seed were collected in the field on March 27, 1958, and tested for germination. One lot of seed was taken from *C. approximata* capsules still attached to the plants. The capsules were off the ground, and while they were slightly damp when collected, they were not wet. Apparently these capsules had been moistened and dried several times during the winter when it rained or snowed; however, the seeds within the capsules were protected from the storms. A second lot of seed was collected that had been produced by the same dodder plants but the capsules were mostly broken and the seed had fallen to the ground during the winter. These seeds were wet from rains and melting snow. Seed collected from dry capsules averaged 6 per cent germination, and those collected from the wet ground averaged 48 per cent germination. Practically all the seed that germinated did so in 4 or 5 days, and the seedlings were normal.

Table 4. Germination on blotting paper in petri dishes of *C. approximata* seed in 16 days at 72° F. following various treatments, 1957.

Treatment before chilling	Treatment after chilling	Percent germination					
		Seed dry during chilling			Seed wet during chilling		
		Days chilled			Days chilled		
		4	14	28	7	14	28
None	None	0	0	0	32	3	10
None	Scarified	8	0	2	1	2	4
None	H_2SO_4 1 hr.	7	7	3	0	3	0
Scarified	None	–	–	–	5	56	68
H_2SO_4 5 min.	None	–	–	–	72	73	91
15 min.	None	–	–	–	73	82	58
30 min.	None	–	–	–	86	70	41 (34)[a]
60 min.	None	–	–	–	57	26	4 (7)[a]
120 min.	None	–	–	–	69	63	18 (64)[a]

[a]Abnormal sprouts.

Acid, dry versus wet chilling and days chilled. Data were obtained from a number of experiments on the effect of the duration of the acid treatment and days chilled on percentage germination of seeds of *C. approximata.* In one experiment some treatments were added to determine if dry chilling of the seed had any effect on dormancy. Data from this experiment appear in Table 4. Low germination was obtained except where the seed had been either scarified or acid treated before a wet chill period.

Optimum time for the acid treatment appeared to vary with the days chilled. A longer treatment in acid seemed to be required with a shorter chill period. A long acid treatment seemed to be harmful where the seed was chilled for the longer periods.

Seeds of different lots of C. approximata need chilling. Germinations were made on 11 collections of seed of *C. approximata* to determine if chilling was a requirement for breaking dormancy in seeds of other lots of this species. These collections were from different areas in the state.

Seed of all lots failed to germinate until they had been treated with acid and chilled. When this was done, some seeds of all lots germinated even though there was variation between lots (Table 5).

Table 5. Germination of *C. approximata* seed collected at several localities, 1958.

Treated in H_2SO_4 minutes	Days chilled at 40°F	Percent germination										
		Location where seed collected and year produced										
		Delta			Deseret	Hinkley	Lyndell	Hyrum			Box Elder Co.	
		1956	1957	1958	1958	1958	1958	Lot 1 1957	Lot 2 1957	Lot 5 1957	Lot 3 1957	Lot 4 1957
0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
5	5	1	2	2	0	0	0	6	2	3	0	3
5	10	30	28	45	9	35	36	27	32	36	8	28
5	20	60	38	53	38	49	43	38	70	55	10	43
5	30	56	39	48	22	47	46	35	61	37	10	54

Interrelation between acid treatment, temperature during chilling, and days chilled. An experiment was run to evaluate the interrelation of the duration of the acid treatment, temperature at which chilling takes place, and the days of chilling required. Two lots of seed were used, one collected in 1956 and the other in 1958. Germination percentages obtained for the various treatments on the two lots of seed were similar except the 1958 seed gave consistently lower germination in all cases than the 1956 seed. Germination percentages for the 1956 seed, for the various treatments, are shown in Figure 2. Untreated seeds did not germinate. Some germination occurred with chilling even without the acid treatment. A five-minute acid treatment increased the germination over no acid, and the thirty-minute acid treatment was distinctly more effective than the shorter time. Temperatures from 35° F to 45° F seemed adequate for chilling. A thirty-minute acid treatment with a ten-day

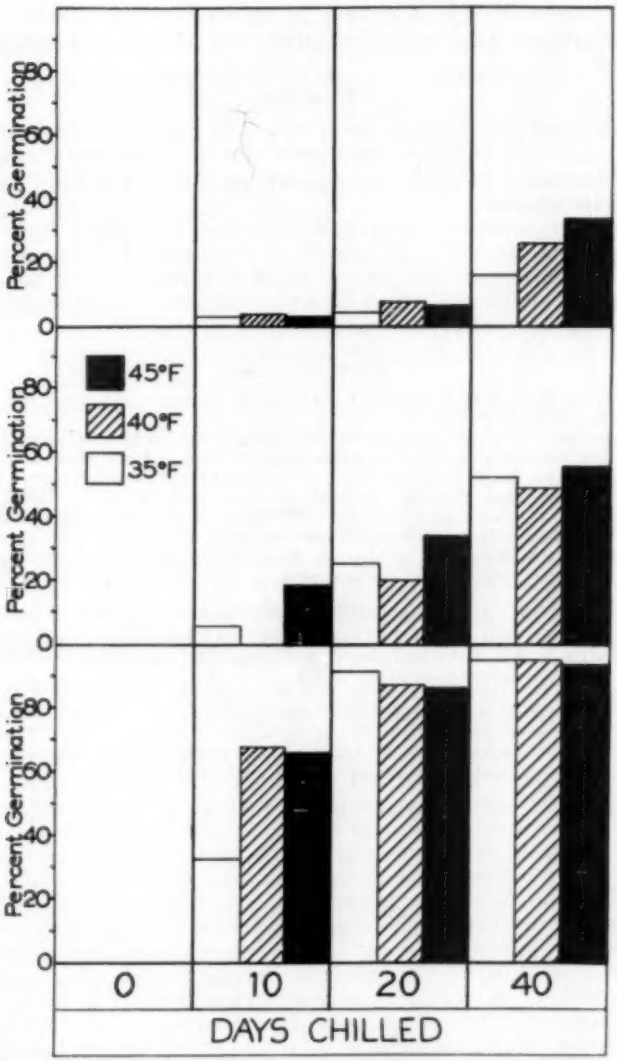

Figure 2. Percentage of seeds of *C. approximata* germinating in 10 days, as related to length of acid treatment, days chilled and temperature during chilling. (Upper no acid, middle 5 minutes acid, and lower 30 minutes acid).

chill period gave about as high percentage germination as a 40-day chill period with a five-minute acid treatment. The highest germination was obtained with a thirty-minute acid treatment and

twenty or more days of chilling at any of the temperatures used. These treatments should give satisfactory results with most lots of seed.

SUMMARY

The usual procedure of breaking dormancy in dodder seeds by either acid treatment or scarification was not effective in breaking seed dormancy in seeds of small-seeded alfalfa dodder *(Cuscuta approximata Bab.)*.

However, dormancy in seeds of this species was readily broken by wet chilling the seed at 35° to 45° F for a period of two to three weeks following the acid or scarification treatment. Acid has been consistently more satisfactory than scarification for making the seeds of *C. approximata* permeable to water.

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Effect of 3–Amino–1,2,4–triazole and L–Histidine on Rate of Elongation of Root Hairs of *Agrostis alba*[1]

Wm T. Jackson[2]

Introduction

It was discovered in 1952 that 3–amino–1,2,4–triazole (amitrole) possesses defoliating and regrowth inhibiting properties in cotton (3). Subsequent study has shown that its most dramatic effect is in the inhibition of chlorophyll formation. Since it is readily absorbed by roots, is translocated throughout the plant, is a general growth inhibitor, and is effective in low concentrations (4), it is a potentially valuable systemic herbicide. It has been used successfully in the control of quackgrass (10), nutsedge (2), Canada thistle (8), and such woody plants as poison ivy.[3]

The mechanism of action of amitrole in bringing about various plant responses is still not understood. It has been established that its action can be antagonized by purines, pyrimidines, histidine, and riboflavin (1, 5, 11).

Hilton (5) found that amitrole at relatively low concentrations inhibits the growth of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. This inhibition could be largely reversed by the addition of *L*–histidine to the medium. The inhibition could not be reversed to the same extent by *D*–histidine, *L*–histidinol, urocanic acid, other *L*–amino acids, purines or pyrimidines. Although he was able to demonstrate the protective effect of *L*–histidine against amitrole toxicity in the two species of yeast, he was not able to do so with one species of bacteria or with seedlings of higher plants. Hilton[4] found that the seedlings exhibited no *visible* symptoms of injury until a week or so after application of amitrole. Therefore, it is possible that *L*–histidine was not able to reverse amitrole-induced inhibition of growth of seedlings because the exogenous supply of *L*–histidine was not available at the time amitrole began to exert its inhibitory effects. A higher-plant growth system in which amitrole-induced inhibition could be detected within a few hours after application would be expected to be more suitable than those requiring several days or weeks for assessing the ability of *L*–histidine to reverse the amitrole-induced inhibition of growth. Jackson (7) has found that the uniform rate of elongation of root hairs of redtop (*Agrostis alba* L.) seedlings can be altered rapidly by application of extremely low concentrations of indoleacetic acid. This system has been employed in the present work to study the effects of amitrole, *L*–histidine, and mixtures of these on the process of cell elongation

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[3]Meyers, W. A., Am. Chem. Paint Company, Tech. Service Data Sheet H–60 on Weedazol, p. 12, May 17, 1955.

[4]Dr. James L. Hilton, personal communication.

for a two hour period immediately following application of the test solution.

METHODS

The method consists essentially in determining, by use of a horizontal compound microscope equipped with an ocular micrometer, the rate of elongation of selected root hairs of redtop seedlings growing in a culture chamber with roots immersed in a simple inorganic solution at pH 5.0–5.1. Although the initial stages of hair formation are quite slow, once the hair has attained a length of 30 to 50 microns elongation continues at a rapid and uniform rate for several hours. Draining and refilling the culture chamber with the same solution does not alter the rate of elongation of hairs. The selected hairs were first measured at 30 minute intervals for a one hour control period. The solution was then drained and replaced with the same inorganic solution plus the test compound or compounds, adjusted to pH 5.0–5.1, an initial measurement made and measurements continued at 30 minute intervals for a two hour treatment period. The rate of elongation of each hair was then calculated for each 30 minute treatment interval as a percentage of the rate of elongation of this same hair during the control period. Each experiment, involving measurement of 5 to 10 root hairs, was replicated at least three times. The methods have been described in detail elsewhere (6, 7). Since light-inactivation of the test compound was not a problem, the microscope lamps were simply equipped with a heat-absorbing filter and the room lighted by two four-foot cool white standard fluorescent tubes. All experiments were performed at a temperature of 21 ± 1° C.

RESULTS

Response of root hairs to amitrole.

10^{-5} to 10^{-1}M amitrole had little or no effect on the rate of elongation of root hairs during the first hour of treatment (Table 1). During the second hour, and particularly during the second half of this hour, both 10^{-2} and 10^{-1}M amitrole were strongly inhibitory, 10^{-3}M slightly so, and 10^{-4} and 10^{-5}M still without effect. Since the

Table 1. Effect of amitrole on elongation of root hairs of redtop seedlings. Growth during the indicated period at each concentration is expressed as a percent of the growth of these same hairs for the one hour period immediately prior to treatment. Standard errors of means are also given for the total treatment period.

Concentration of amitrole M	Per cent elongation for various time periods minutes after initiation of treatment				
	0–30	0–60	0–90	0–120	90–120
0	51	103	155	206 ± 3.0	51 ± 1.3
10^{-1}	51	102	127	137 ± 10.6	10 ± 1.8
10^{-2}	49	91	117	136 ± 10.0	18 ± 2.8
10^{-3}	45	96	142	184 ± 8.4	43 ± 3.2
10^{-4}	46	103	156	205 ± 8.2	48 ± 2.1
10^{-5}	42	98	157	206 ± 10.4	49 ± 3.4

inhibition induced by 10^{-2}M amitrole was as great as that induced by 10^{-1}M, 10^{-2}M amitrole was selected as the most appropriate concentration to use in combination with *L*–histidine to determine whether or not *L*–histidine at any concentration could, in whole or in part, overcome the inhibitory effects of amitrole.

These results showed that the degree of inhibition of root hair elongation increased with time and suggested that 10^{-3}M amitrole would cause strong inhibition of growth if the treatment period could have been extended for another hour. Unfortunately, the root hairs become so long during the third hour following treatment that they exceed the length of the ocular scale, thus making further measurements of dubious accuracy.

Response of root hairs to L–histidine.

During the first hour of treatment 10^{-2} to 10^{-5}M *L*–histidine had little or no effect on the rate of elongation of root hairs, whereas 10^{-1}M *L*–histidine stopped growth immediately (Table 2). However, during the last half of the second hour of treatment, the rate of

Table 2. Effect of *L*–histidine on elongation of root hairs of redtop seedlings. Growth during the indicated period at each concentration is expressed as a percent of the growth of these same hairs for the one hour period immediately prior to treatment. Standard errors of means are also given for the total treatment period.

Concentration of L-histidine M	Per cent elongation for various time periods, minutes, after initiation of treatment				
	0–30	0–60	0–90	0–120	90–120
0	51	103	155	206 ±3.0	51 ±1.3
10^{-1}	0	0	0	0 ±0.0	0 ±0.0
10^{-2}	56	107	149	179 ±7.0	31 ±2.3
10^{-3}	50	92	131	166 ±9.0	35 ±4.0
10^{-4}	48	93	138	181 ±8.4	43 ±3.7
10^{-5}	46	96	148	198 ±9.2	50 ±3.6

elongation of hairs subjected to 10^{-2}M *L*–histidine was reduced to about 60% of the control, 10^{-3}M to 68%, and 10^{-4}M to 85%. Since 10^{-1}M *L*–histidine stopped growth immediately, it was even more toxic than 10^{-1}M amitrole during the first two hours of treatment. The effect of 10^{-1}M *L*–histidine was probably non-specific.

Response of root hairs to mixtures of amitrole and L–histidine.

The effect of 10^{-1} to 10^{-5}M *L*–histidine in combination with 10^{-2}M amitrole on the rate of elongation of root hairs was determined for a two hour treatment period (Table 3). The cessation of growth in 10^{-2}M amitrole plus 10^{-1}M *L*–histidine was immediate. This was undoubtedly due principally to *L*–histidine. The presence of 10^{-2} to 10^{-5}M *L*–histidine did not alter significantly the response of root hairs to 10^{-2}M amitrole during the first hour of treatment. However, during the last half of the second hour, 10^{-3} to 10^{-5}M *L*–histidine did nullify partially the inhibitory effects of amitrole. Compared to the rate of elongation of root hairs in 10^{-2}M amitrole alone during the period 90–120 minutes after treatment, the rate in 10^{-2}M

Table 3. Effect of 10^{-2}M amitrole and the indicated concentrations of *L*–histidine on elongation of root hairs of redtop seedlings. Growth during the indicated period at each concentration is expressed as a percent of the growth of these same hairs for the one hour period immediately prior to treatment. Standard errors of means are also given for the total treatment period.

Concentration of L-histidine M	Per cent elongation for various time periods, minutes, after initiation of treatment				
	0–30	0–60	0–90	0–120	90–120
10^{-1}	0	0	0	0± 0.0	0±0.0
10^{-2}	48	88	116	133± 7.2	17±1.9
10^{-3}	45	87	121	141± 9.8	20±2.2
10^{-4}	48	96	135	160±10.8	26±2.9
10^{-5}	42	81	112	138± 8.2	26±2.6
0	49	91	117	136±10.0	18±2.8
Control[a]	51	103	155	206± 3.0	51±1.3

[a]Neither amitrole nor L-histidine present.

amitrole plus 10^{-3}M *L*–histidine was 108%, in 10^{-2}M amitrole plus 10^{-4}M *L*–histidine it was 142% and in 10^{-2}M amitrole plus 10^{-5}M *L*–histidine the rate was 144%.

DISCUSSION

Since the discovery that amitrole possesses defoliating and regrowth inhibiting properties (3), much information has accumulated on its effects on various biochemical systems and upon developmental aspects of plant growth such as plastid formation. Relatively little is known about its effects on the process of cell elongation, and even less about the mechanism by which it exerts these effects. It should be mentioned that Hall et al. (4) have studied the interaction of amitrole and indoleacetic acid in controlling coleoptile section elongation and that Racusen (9) has postulated that the lowering of catalase activity in leaves subjected to amitrole may be a clue to its toxicity in plants.

The recent finding (1, 5, 11) that the inhibitory action of amitrole can be overcome by simultaneous applications of purines, pyrimidines, histidine, and riboflavin suggest that it may exert its effect through blocking of metabolic pathways involved in the synthesis of nucleic acids and proteins. Of particular interest in relation to the present study is the demonstration by Hilton (5) that 3 to 4 × 10^{-5}M amitrole causes a 50-percent inhibition of *Saccharomyces cerevisiae* growth and that the addition of 10^{-2}M *L*–histidine to the growth medium causes a 3,000-fold increase in the amount of amitrole required to inhibit growth to the same extent in the same organism. Histidine alone had no effect on yeast growth; but the protective effect of *L*–histidine against amitrole toxicity was apparent at histidine concentrations as low as 10^{-6}M; and 10^{-4}M was almost sufficient to produce the maximum effect. The striking effect produced by *L*–histidine could not be duplicated with even 10^{-3}M concentrations of other *L*–amino acids or with purines or pyrimidines. If the amitrole concentration were low enough, 2 × 10^{-4}M or lower, other amino acids did reverse this inhibition. He did find slight reversal

of amitrole inhibition by, in order of decreasing effectiveness, cytosine, guanine, adenine, hypoxanthine, 4–amino–5–imidazole carboxamide and uracil. The striking effects of *L*–histidine led him to study its action in cultures of a wild strain and five different histidine-requiring mutant strains of *Schizosaccharomyces pombe*. The wild strain responded in a manner similar to that of *Saccharomyces cerevisiae*. However, 10^{-4} to 10^{-2}M amitrole did not inhibit growth of the histidine-requiring mutant strain in the presence of 10^{-5} to 10^{-4}M *L*–histidine. These results suggest that amitrole does not prevent the *utilization* of *L*–histidine. Hilton (5) also attempted to determine whether the addition of *L*–histidine could offset the action of amitrole in higher plants. Several different techniques were used in applying amitrole and *L*–histidine to seedlings of wheat, oats, barley, and tomatoes. There was no evidence that addition of *L*–histidine could nullify the action of amitrole. Hilton[4] stated that amitrole did not induce visible effects on the above seedlings for a week or so and that perhaps the plants were being slowly deprived of some growth factor. Since results of the present investigation demonstrate that 10^{-2}M amitrole will reduce the rate of elongation of root hairs to 35% of the control rate in the period 90–120 minutes following treatment and that root hairs treated simultaneously with 10^{-2}M amitrole plus 10^{-5}M *L*–histidine grow 51% as rapidly as the controls, it is apparent that (a) amitrole-induced reduction in growth of root hairs is rapid and (b) partial reversal of this inhibition by *L*–histidine is also rapid. However, it should be emphasized that although a higher concentration of amitrole, 10^{-2}M, was required to inhibit root hair elongation in this short period than to inhibit yeast growth over a longer period, the same concentration of *L*–histidine, 10^{-4}M, was effective in reversing the amitrole-induced inhibition. These findings are consistent with the hypothesis of Hilton (5) that one action of amitrole is to block the synthesis of *L*–histidine. These results plus the demonstration (1, 11) that amitrole-induced inhibition can be overcome by purines and pyrimidines and riboflavin suggest that amitrole is able to exert its inhibitory effects in several ways.

SUMMARY

The rate of elongation of root hairs of *Agrostis alba* seedlings is virtually unaffected by 10^{-5} to 10^{-1}M 3–amino–1,2,4–triazole (amitrole) during the first hour of treatment. During the second hour, and particularly during the second half of this hour, the rate of root hair elongation is strongly reduced by 10^{-2} and 10^{-1}M amitrole. The inhibition induced by 10^{-2}M amitrole can be partially overcome by the simultaneous addition of 10^{-5} to 10^{-3}M *L*–histidine. Compared to the rate of elongation of root hairs in 10^{-2}M amitrole during the period 90–120 minutes after treatment, the rates in 10^{-2}M amitrole plus 10^{-3}M, 10^{-4}M, and 10^{-5}M *L*–histidine were respectively 108%, 142%, and 144%. These findings are consistent with the hypothesis that amitrole is blocking one or more steps in the synthesis of *L*–histidine.

[4]Dr. James L. Hilton, personal communication.

ACKNOWLEDGMENTS

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The Decomposition of Amitrole in California Soils[1]

B. E. Day, L. S. Jordan, and R. T. Hendrixson[2]

Citrus and avocado trees have occasionally been injured following use of 3–amino–1,2,4–triazole (amitrole) at rates of 12 or more lb/A for control of perennial weeds. The distribution of tree injury relative to sprayed areas as well as the known low volatility of amitrole (1) has indicated that tree injury was not due to volatility. The initial occurrence of symptoms on sucker growth in the interior and on the top foliage rather than lower exterior foliage of the trees would seem to eliminate spray drift as a possible cause. This injury has appeared to be due to spray residues being leached into the soil and taken up by the roots of the trees.

Instances of injury to row crops by persistent residues of amitrole have also been observed. However, there is much experience to indicate that the persistence of amitrole in such cases is exceptional. In California, such persistence has been more frequent in some areas than in others, suggesting that differences related to climate or soil may be responsible.

Amitrole has been used primarily as a foliar herbicide and has shown little promise as a soil treatment. Consequently, there has been little research on its herbicidal properties in soils. However, the initial and residual phytotoxicity is known to vary among soils (3, 4, 5). In seven soils studied, Sund (4) determined by analysis of aqueous extracts that 10 to 70 percent of added amitrole was adsorbed on the soil colloids. This initial adsorption as well as the rate of decomposition of amitrole over a period of time was directly correlated with the base exchange capacity of the soil. Toxicity to tomato plants was in direct proportion to the amounts of amitrole recoverable from the soil by extraction with water. These data along with the ability of the herbicide to form stable complexes with metals led Sund to propose that the disappearance of amitrole from soils might be due to a combination of complexing and adsorbing actions.

The objective of the present study was to secure further information on the mode of deactivation of amitrole and the nature of the factors affecting its disappearance from soils as related to the use of amitrole in orchards and in fields prior to the planting of crops.

Methods

Fifty-five soils were collected from the principal citrus areas of California for a survey of their capacity to adsorb and decompose amitrole. With the exception of the muck, collected from the Sacramento delta, the soils were selected as representative of the range of soil conditions of subtropical fruit orchards. Twenty-six of the

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soils were collected from citrus orchards in which base exchange capacity and other soil characteristics had been determined by Harding (2). Samples of surface soil from several locations in a field were mixed thoroughly. The samples were then taken from the composite, air-dried, and screened to remove stones and roots.

More detailed studies of some of the factors affecting amitrole decomposition were made for the following five soils: Chino silt loam, a dark brown alluvial soil of the valley floors; Diablo clay, a black soil of the coastal hills, residual from limestone; Hanford sandy loam, a light-textured soil formed by recent granitic alluvial deposits; Ramona loam, a medium-textured terrace soil of older alluvial granitic material having moderate profile development; and Vista sandy loam, a light-textured, upland soil residual from granite. These five soils are typical of the southern California coastal and intermediate valley areas and in addition cover a breadth of conditions as to texture and derivation.

Phytotoxicity.

The phytotoxicity of amitrole in the five test soils was determined over a range of concentrations using successive plantings of sesbania and sudangrass as test plants. The concentrations of amitrole were initially set in a logarithmic series at 0.5, 1, 2. . .32 ppm in 400 gm of air-dry soil. Wax-treated paper cups without drainage holes were used as soil containers. Sesbania and sudangrass were planted at intervals of 0, 5, 15, and 29 days after addition of the herbicide to the soil. Moisture levels were maintained in the range of 50 to 90 percent of field capacity by weighing the cultures and adding deionized water daily. Twenty-one days after planting, the plant shoots were harvested, oven-dried, and weighed. Final ratings of the factors, height, visual estimate of injury, and dry weight of plant material expressed as percent of the untreated control were averaged and are reported as "Index of Plant Growth."

Chemical analyses.

Known amounts of amitrole were added to samples of the soils and the initial recovery and rates of decomposition determined by immediate and subsequent analyses. Analyses were by a modification of the method of Sund (4). Minor changes in volumes and aliquots were made in the method to adapt it to use with the Bausch and Lomb "monochromatic" colorimeter and the pH of the nitroprusside reagent was adjusted to 10.5 rather than 11.0 as in the original method. Otherwise, the method was identical to the one reported by Sund.

A standardized technique was employed in preparing the soil samples and making the extracts for analysis. Such routines as stirring and filtering, as well as the standing time between procedures, were carried out according to a uniform time schedule for all experiments. Samples of 100 gm of air-dry soils were weighed into polyethylene cups and moistened with a solution containing 5 mg or 20 mg of amitrole in distilled water. Samples were held at constant temperatures (30° C except in experiments in which temperature was studied

as a variable) in humid seed germinators during the incubation period. Soil moistures were maintained constant by periodic addition of water to maintain the cultures at constant weight.

Extracts for analysis were made by adding sufficient water to each cup to bring the total weight of soil and water to 200 gm, the mixture then being equal parts of soil and water. After stirring for 1 minute, and separation by gravity filtration, samples of adequate volume were drawn from the total filtrate and centrifuged to clarity if needed. An analysis was thus not a measure of the total amitrole present but was a determination of the amount in solution excluding the amitrole adsorbed on the soil colloids. There is evidence (4) that this is a more valid measure of the amitrole available to cause plant injury than would be obtained by more complete extraction methods.

Control cultures, differing only in not having the amitrole added, were carried through all procedures at the same time and under the same conditions as the amitrole-treated cultures. The color blanks obtained were used to adjust the slit of the colorimeter to 100 percent transmission, thus effectively canceling differences due to traces of turbidity or spurious color development.

Factors studied.

The factors of time, mixtures of soils, steam sterilization, temperature, soil moisture, leaching, adsorptive capacity of the soil, and relationship to base exchange capacity were studied.

Rates of leaching of amitrole were determined by applying the herbicide to the surface of soil columns and leaching with distilled water. Glass columns 4 cm in diameter were uniformly packed with 400 gm of soil or quartz sand. Fifty mg of amitrole in 1 ml of water were added to the soil surface and leached into the soil with two successive 5-ml additions of water. Water was then continuously added at such a rate as to maintain a 5- to 10-mm head of water above the soil surface. The leachings were collected in successive units of 10 ml and analyzed for amitrole.

In those experiments in which soil-moisture levels below field capacity were employed, the herbicide was added as a powder and thoroughly mixed with the soil before addition of the water. The problem of obtaining uniform distribution of low levels of moisture was overcome by slowly adding the water as a fine spray while rapidly tumbling the soil by a motor-driven rotating device.

In the survey of amitrole depletion in 55 soils, 5 mg of amitrole were added to 100-gm samples of the air-dried soils and analyses made both immediately and after 14 days. Through the full range of soils, no apparent difficulty was encountered in the extraction or other technical details of the analyses. Filtration of some of the soil mixtures was slow, but in all cases a clear extract suitable for analysis was obtained.

All cultures and analyses including the controls were replicated four times except that two replicates were employed in the general survey of amitrole decomposition in the full range of soils.

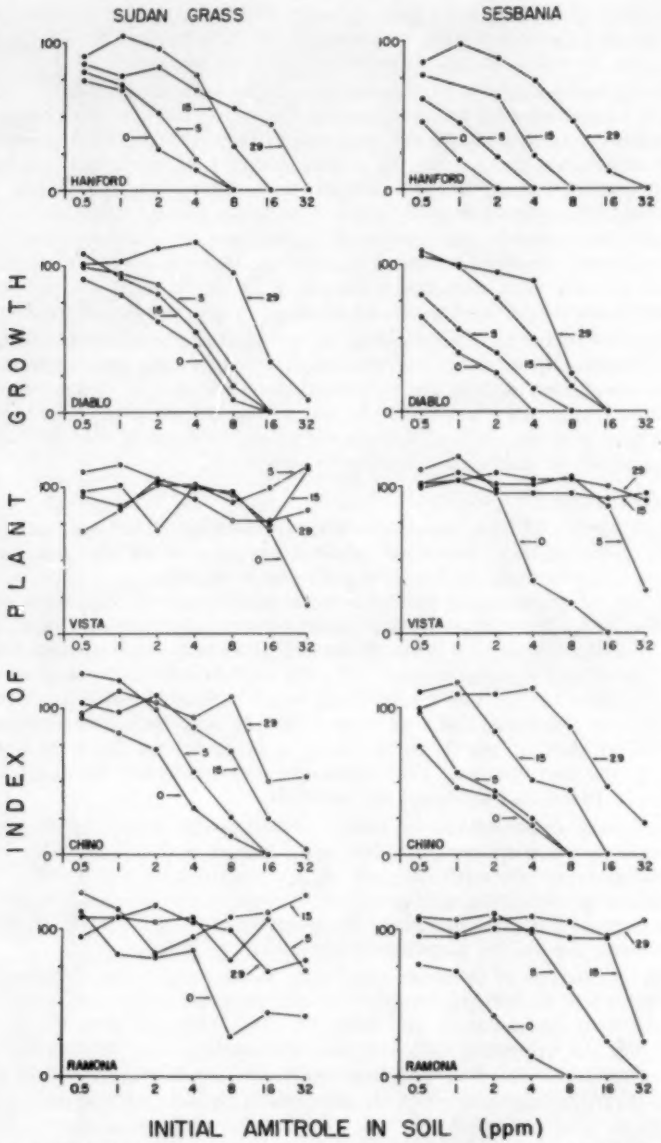

Figure 1. Growth response of a series of plantings of sudangrass and sesbania in five soils treated with seven concentrations of amitrole. The numbers on the lines refer to the number of days elapsed after treatment of the soil with amitrole until planting of the seeds.

RESULTS

Phytotoxicity.

The data on the phytotoxicity of amitrole as affected by planting delay are given in Figure 1. Sudangrass was less sensitive than sesbania to the presence of amitrole. This may be due in part to the 4-day germination time of sudangrass allowing more time for decomposition of the amitrole after planting than the 2 days required for germination of sesbania. The growth of sudangrass was more variable than the growth of sesbania and the apparent response to amitrole was correspondingly more erratic. Initial amitrole toxicity differed greatly among the five soils. Rate of detoxification of amitrole as indicated by the response of the delayed plantings was rapid but not uniformly so.

Chemical analyses.

The recovery of amitrole initially and weekly over a period of two to four weeks from the five principal test soils and a mixture of two of these soils is given in Figure 2. The initial amitrole levels were 20 mg per 100-gm soil sample and moisture levels were maintained at 90 per cent of field capacity. The soil mixture was composed of 95 parts of Chino silt loam soil and 5 parts of Vista sandy loam.

The amount of amitrole recovered initially ranged from 73 percent in the Chino silt loam to 97 percent in Hanford sandy loam. Rates of depletion of amitrole were approximately linear varying from 0.3 mg per week for Hanford to 10 mg per week for Vista—a difference of more than thirtyfold. The soil mixture was included to determine whether or not a small amount of soil possessing the ability to rapidly decompose amitrole could serve to "inoculate" soil

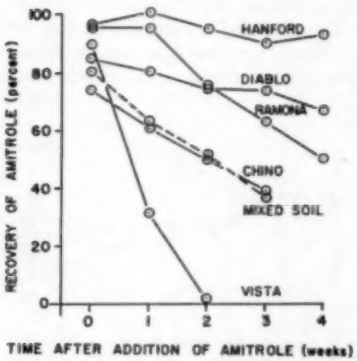

Figure 2. Recovery of amitrole from five soils and a mixture of 95 per cent Chino and 5 per cent Vista soils expressed as per cent of the 20 mg originally added per 100 gm of air-dried soil.

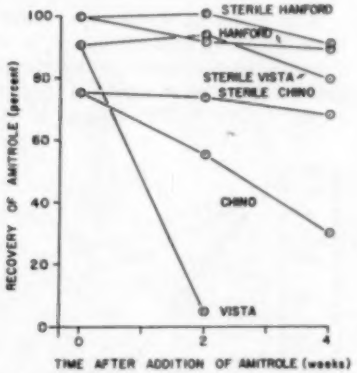

Figure 3. Recovery of amitrole initially and two and four weeks after addition to autoclaved and unsterilized samples of three soils.

in which amitrole decomposition was relatively slow. In the composite sample, amitrole was depleted at the rate of 2.7 mg per week as compared with 2.2 mg per week for Chino soil alone. The rate of 2.6 mg per week would be expected on the basis of the proportions of soils used. With this small difference, no "inoculation" effect can be considered to have occurred. The depletion rates obtained by these analyses confirm the data from the plant responses in the delayed plantings (Figure 1) with the exception of Hanford sandy loam for which the biological data indicate a more rapid breakdown than do the analyses.

Soil sterilization.

The effect of sterilization by steam on the rates of depletion of amitrole in three of the soils is shown in Figure 3. The soils were autoclaved in metal cans, transferred to clean (but not sterilized) polyethylene cups, and 20 mg of amitrole were added to each sample in sufficient distilled water to raise the soil moisture level to 90 percent of field capacity. Sterilization did not affect the amount of amitrole immediately recoverable from the soils. Sterilization greatly reduced the rate of decomposition of the herbicide over the four-week period in the Vista and Chino soils, but had little effect on the slow rate of decomposition in Hanford soil. Breakdown in the Hanford soil either with or without sterilization was about the same as in the other two soils after sterilization. This indicates that the relatively high rates of disappearance of amitrole in the Chino and Vista soils are due predominantly to biological action. A low rate of decomposition of amitrole, or possibly a slow adjustment of adsorption equilibrium, occurs in the soils independently of biological factors.

Soil temperature.

The effect of soil temperature on rates of decomposition of amitrole by Vista and Chino soils was determined as shown in Figures 4 and 5. Initial amitrole levels were 20 mg per 100-gm sample of soil and analyses were made at four-day intervals on samples maintained at 10° C, 20° C, 30° C, and 40° C.

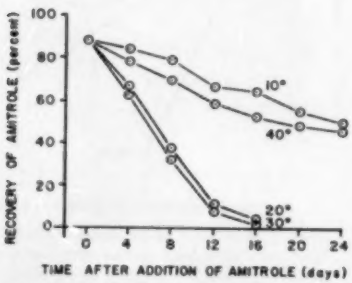

Figure 4. Recovery of amitrole from samples of Vista sandy loam at four temperatures.

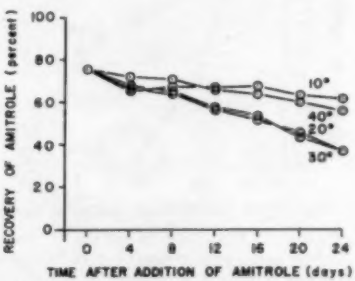

Figure 5. Recovery of amitrole from samples of Chino silt loam at four temperatures.

Amitrole was broken down most rapidly at intermediate temperatures. In each of the soils, decomposition was much slower at both high and low temperatures, with an optimum temperature between 20° C and 30° C. This pattern of response further supports the view that amitrole depletion is predominantly a biological action.

Soil moisture.

The effect of soil moisture on recovery of amitrole from Vista, Chino, and Diablo soils was determined as shown in Figures 6, 7, and 8. Moisture was maintained at five levels from 16 per cent to 80 per cent of the field capacity of the individual soils. The initial amitrole concentration was 20 mg per 100-gm soil culture.

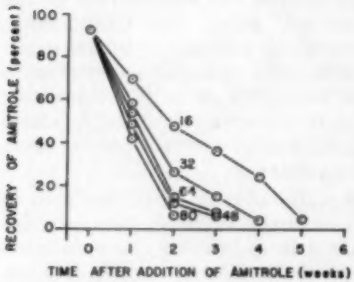

Figure 6. Recovery of amitrole from samples of Vista sandy loam at 30° C and five moisture levels. The numbers on the curves refer to moisture levels in per cent of field capacity.

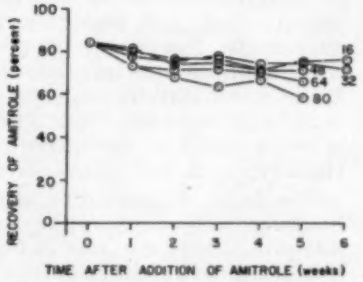

Figure 8. Recovery of amitrole from samples of Diablo clay at 30° C and five moisture levels. The numbers on the curves refer to moisture levels in per cent of field capacity.

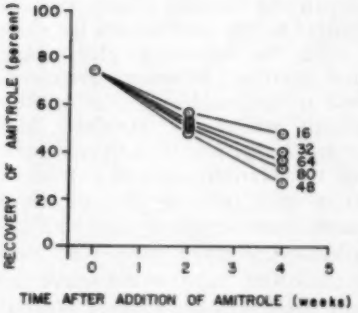

Figure 7. Recovery of amitrole from samples of Chino silt loam at 30° C and five moisture levels. The numbers on the curves refer to moisture levels in per cent of field capacity.

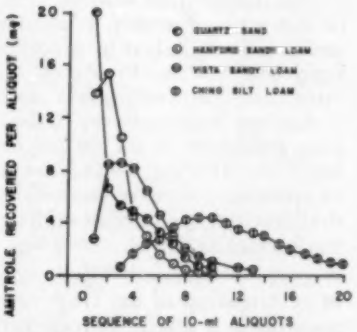

Figure 9. Recovery of amitrole from successive, 10 ml increments of distilled water leached through cylinders 4 cm in diameter containing 400 gm of soil or quartz sand. Surface application of 50 mg of amitrole was made before leaching.

The data show that amitrole decomposition occurred over the full range of soil moistures tested. For Vista and Chino soils (Figures 6 and 7), there was appreciable delay in amitrole decomposition at the 16 per cent moisture level with little correlation between decomposition and moisture levels above 48 per cent of field capacity. For the Diablo soil (Figure 8) throughout the series of periodic analyses, there was a rough positive correlation between moisture content and amitrole decomposition.

Leaching.

The data on leaching are given in Figure 9. It is apparent that amitrole moves readily with the leaching water but with distribution patterns that vary appreciably among soils. For quartz sand, the peak concentration was in the first 10 ml sample but was delayed to the second, third, and tenth for Hanford, Vista, and Chino soils, respectively. This order is the inverse of the per cent of initial recovery of amitrole from mixtures of these same soils and water (Figure 2) and is thus directly related to the adsorptive capacity for amitrole of the soils and sand. These adsorptive capacities in equal mixtures of soil or sand with water were 0, 3, 11, and 27 per cent for the sand, Hanford, Vista, and Chino soils, respectively.

The depth of penetration of the water from a particular rain or irrigation following spraying with amitrole becomes the basis for interpretation of the data in Figure 9 in estimating the concentration of amitrole at different depths in the soil. For example, if leaching water were to penetrate to a depth of 20 units (inches, cm, or mm) relative concentrations for the different soils could be estimated directly from the curves for each unit of depth. Zero on the horizontal axis in Figure 9 would be the moving front of the leaching water and 20 on the same axis would be the soil surface.

The distribution is in all cases relative to the depth of movement of the water. For any particular depth the vertical distribution of amitrole is described by a curve similar to the ones shown for each particular soil in Figure 9, but with the horizontal axis either "stretched" or "compressed" to equal the depth of water movement.

Average total recovery of amitrole in the leaching water for the four replicates of the 50 mg originally added was: Hanford, 54.6 mg; Vista, 46.5 mg; and Chino, 46.0 mg. In spite of the obvious error in apparent excessive recovery from the Hanford soil, it is evident that leaching by rainfall or irrigation water removes amitrole from the surface layers of soil. Thus leaching could assure that residues of preplanting applications of amitrole are taken below the zone of germination of the crop, or on the other hand, could move the amitrole as a relatively concentrated band into the zone of greatest root density of orchard trees. This evidence is also important in the interpretation of the results obtained by the extraction methods employed in the analyses reported in this paper. It is clear that the amitrole not recovered in the water phase is in fact reversibly bound by the soil in some manner and not destroyed or permanently bound.

Depletion in various soils.

Data for the survey of amitrole depletion in 55 California soils are given in Table 1.

Three soils collected from the Coachella Valley gave apparent initial recoveries of amitrole significantly in excess of the amount added. The apparent error could be eliminated only by use of colorimeter curves standardized specifically for each of the soils. For the sake of uniformity, data are given on the basis of a common standard curve. Consequently, initial recovery of amitrole from two

Table 1. Average per cent recovery of amitrole initially and after two weeks from California soils.

Map code	Soil classification	Location	Per cent recovery	
			Initial	Two-week
19	Hanford stony sandy loam	Pauma	74	0
20	Hanford loamy sand	Pauma	86	40
23	Hanford gravelly sandy loam[a]	La Verne	90	0
17	Hanford sandy loam[a]	Highgrove	88	16
18	Hanford sandy loam[a]	Highgrove	79	45
13	Hanford sandy loam[a]	Arlington	68	0
52	Hanford sandy loam[b]	Mentone	97	94
49	Bautista sandy loam	Hemet	96	Trace
27	Fallbrook fine sandy loam	Fallbrook	74	40
51	Vista sandy loam[b]	Fallbrook	89	8
48	Vista sandy loam[b]	Escondido	89	0
1	Vista sandy loam[a]	Escondido	99	27
2	Vista sandy loam[a]	Escondido	74	11
3	Vista sandy loam[a]	Escondido	92	0
14	Greenfield sandy loam[a]	Riverside	92	24
15	Greenfield sandy loam[a]	Riverside	97	23
16	Greenfield sandy loam[a]	Arlington	96	32
22	Greenfield sandy loam[a]	Highland	88	Trace
6	Sorrento fine sandy loam	Goleta	89	Trace
7 A	Elkhorn loamy sand	Encinitas	90	8
7 B	Elkhorn loamy sand	Encinitas	83	14
24	Superstition very stony sand	Coachella	109	60
25	Superstition stony sand	Coachella	108	79
26	Coachella very fine sand	Coachella	106	28
8	Indio loam	Coachella	98	35
9	Indio clay loam	Coachella	94	37
10	Las Posas loam	Fallbrook	88	37
21	Ramona loam[a]	Mentone	80	0
11	Ramona loam	Riverside	84	0
53	Ramona loam[b]	Riverside	96	76
4	Yolo sandy loam[a]	Capistrano	88	0
5	Yolo loam[a]	Santa Ana	54	0
28	Yolo loam[a]	Northridge	71	0
30	Yolo loam[a]	Camarillo	79	0
29	Yolo loam, high fan phase[a]	Corona	89	0
31	Yolo loam	Goleta	78	Trace
32	Yolo loam	Ventura	83	Trace
34	Yolo loam	Ventura	50	0
12	Yolo silt loam[a]	Corona	83	0
33	Yolo silt loam[a]	Ojai	87	0
39	San Joaquin sandy loam[a]	Ivanhoe	53	0
40	San Joaquin sandy loam[a]	Ivanhoe	84	0
45	San Joaquin loam[a]	Strathmore	25	0
46	San Joaquin loam	Woodlake	94	0
47	San Joaquin clay loam	Orange Cove	91	26
42	Exeter loam gravelly phase[a]	Strathmore	50	Trace
37	Exeter loam[a]	Exeter	72	0
43	Exeter loam	Lindsay	54	0
38	Hovey adobe clay	Orange Cove	76	0
36	Ducor adobe clay[a]	Porterville	84	0
35	Porterville adobe clay[a]	Terra Bella	81	0
44	Porterville adobe clay	Orange Cove	86	0
50	Chino silt loam	Chino	73	33
54	Diablo clay[b]	Oceanside	84	74
41	Muck	Isleton	19	10

[a]Soils studied by Harding (2).
[b]Virgin soils.

soils of the Superstition and one of the Coachella series are reported in excess of 100 per cent.

The soils in Table 1 are grouped by series and these arranged in the general order of lighter to heavier texture. The arrangement is to some extent geographical since the desert and upland series tend to be lighter in texture than the series from the coastal areas and the San Joaquin Valley.

The most apparent characteristic of the data as a whole is the high degree of variability in the persistence of amitrole among the soils tested. After two weeks, residues were entirely absent in 26 of the 55 soils, present in only trace amounts in 6 others, and present in substantial quantities in the remaining 23. The variability is largely random with respect to soil classification, although there is some trend toward lower residues in the loams and heavier textures reported in the lower part of the table. It is, nevertheless, clearly evident that soil classification, as such, provides an insufficient basis for the practical evaluation of residue persistence. The wide variation among soils of the same classification is seen in the range of residues remaining after two weeks: 0 to 94 per cent among the seven Hanford soils and the 0 and 76 per cent residue values for Ramona loam taken from two sources near Riverside.

The geographical sources of the soils along with classification appear to offer a rough basis for estimating amitrole persistence. The ten samples of Yolo soil, all collected from coastal areas, were consistently free of residue, whereas residues were present in all of the five soils from the Coachella Valley. Both of these results confirm field experience. Use of amitrole in orchards and vineyards has usually caused tree or vine injury in the Coachella Valley but rarely so in the coastal areas. Lower residue hazard in the San Joaquin Valley is shown by the persistence of amitrole in only 1 of the 12 soils collected from that area. These geographical relationships are better seen on the sketch map (Figure 10).

This map shows the locations from which the soils were collected along with a graphical representation of the amitrole residue remaining after two weeks. The numbers on the map refer to the numbers in the left column of Table 1. The muck soil was collected from outside the area shown on the map and is not plotted. An average value is plotted for soils 7A and 7B which were collected from opposite sides of the same orchard. From the map, it is apparent that residue levels tend to be high in the lighter upland and desert soils and low in the heavier soils of the San Joaquin and coastal valleys.

A possible means of correlating amitrole decomposition with soil characteristics is based on the observation by Sund (4) of positive correlation between the rate of depletion and the capacity of a soil to adsorb amitrole and between both these factors and base exchange capacity. If this were generally true, we should expect the amount of amitrole recovered after two weeks to be high from those soils from which initial recovery was high. By inspection of the values in Table 1, it can be seen that this is frequently true but with many

Figure 10. Map of amitrole recovery from southern California orchard soils. The blackened portion of each circular graph placed at the point of collection of the soil indicates the proportion of added amitrole remaining after two weeks at 30° C.

exceptions. The mean initial recovery of amitrole from the 26 soils having no residue after two weeks was 76.2 per cent. Excluding the 6 soils having final residues in the "trace" category, the mean initial recovery was 87.9 per cent from the 23 soils in which amitrole did not fully decompose. Mean values for adsorption are thus 23.8 and 12.1 per cent, respectively, for the two categories of soil. This difference in adsorption is statistically significant at the 5 per cent but not at the 1 per cent level of probability.

The depletion of amitrole in those soils in which residue remained at the two-week analysis may be further examined for correlation with amitrole adsorption. For 19 soils, these factors have a correlation coefficient of 0.176, considerably less than the value of 0.456 for significance at the 5 per cent level. It is evident that amitrole depletion is not closely related to adsorption.

Values for total base exchange capacity for 23 of the soils were available from Harding (2). These values range from 5.1 to 34.2 me/100 gm and have a correlation coefficient with the capacity of the soil to adsorb amitrole of 0.151 (0.413 required for significance). It is evident that the variation of amitrole adsorption is largely independent of base exchange capacity, indicating that the sites and mechanism of adsorption differ from those concerned in base exchange.

Of these 23 soils, breakdown of amitrole was complete in 15 having an average base exchange capacity of 13.6 me/100 gm. A trace of amitrole was present in one soil and measurable residues remained in seven soils having a mean base exchange capacity of 7.9 me/100 gm. The difference of 5.7 units is significant at the 1 per cent level. For the larger number of soils there is a positive relationship between base exchange capacity and the ability of a soil to decompose amitrole. However, the correlation coefficient for these factors for the seven soils having residues was only 0.123 (significance = 0.754).

DISCUSSION

The relationship between the analytical and biological data for the five soils shows that the capacity of the soil to decompose amitrole rather than capacity to adsorb amitrole is the determining factor in both the immediate and the delayed plant response to soil applications of amitrole. It appears that the time required for seeds to germinate and roots to develop is sufficient for the depletion of appreciable quantities of amitrole. Adsorption is of greater significance through its determination of the pattern of leaching of amitrole in different soils, thus governing distribution of amitrole in the root zone.

The pronounced effect of steam sterilization leaves little doubt that soil microorganisms are the main agency in the depletion of amitrole in soils. Adsorption on soil colloids and complexing with metallic ions, the mechanism suggested by earlier work (4), could account for that portion of the disappearance of amitrole observed to be unaffected by steam sterilization.

The highly variable rates of depletion of amitrole in different soils are likely due to differences in populations of soil organisms. These differences are correlated in a general way with soil classification, texture, base exchange capacity, and amitrole adsorption capacity. The relationships in each case are not close and thus do not provide a useful index of the capacity of the soil to decompose amitrole, nor can any particular cause-and-effect relationship be shown. The soils studied range from desert sands having only limited development of the usual chemical, physical, and biological properties of soil to soils with these properties developed to a high degree. The microorganism populations capable of decomposing amitrole follow the same trend.

The data show three discrepancies that are probably related: (1) the disagreement between plant response and analysis in determin-

ing the rate of decomposition of amitrole in Hanford sandy loam, (2) the apparent excessive recovery of amitrole in this same soil as shown by analysis in the leaching experiment, and (3) the apparent excessive recovery of amitrole in the analyses of three desert soils. Seemingly in these cases the analyses show more amitrole present than is actually present. Although the analytical method is not entirely specific for amitrole, control determinations show that the error is not due to a faulty assay of related substances already present in the soil but to some reaction resulting from the addition of the amitrole. A reasonable explanation is that the amitrole reacts in these soils to form a related compound that is more active in the analysis and less phytotoxic than the parent amitrole. This assumption would explain the discrepancies noted and would not seem unreasonable in the light of the unspecific nature of the analysis and the reported capacity of amitrole to react with metallic ions (4).

SUMMARY

Adsorption, phytotoxicity, rates of leaching, and persistence of amitrole as affected by variations in soil type, temperature, moisture, and sterilization by steam were studied in soils collected from California citrus districts. In 55 soils studied, adsorption of amitrole was usually less than 20 per cent of the amitrole added and only rarely greater than 50 per cent. The adsorption was found to be readily reversible, and amitrole could be quantitatively leached from soils by continued percolation with water. The rate of downward leaching of the amitrole relative to the movement of the water was retarded in proportion to the capacity of the soil to adsorb amitrole.

Rates of decomposition of amitrole in steam-sterilized soils were much lower than the rates in unsterilized soils indicating that the decomposition is primarily due to the action of soil microorganisms. The microorganisms concerned have a temperature optimum between 20° C and 30° C. Decomposition of amitrole was slower at low than at medium and high soil-moisture levels.

Rates of decomposition of amitrole were highly variable among the soils studied, apparently because of differences in populations or levels of activity of the soil microorganisms concerned. Amitrole decomposition was not closely related to soil classification, texture, base exchange capacity, or amitrole adsorption capacity, although there was some correlation with all of these factors.

A short residue life for amitrole was more frequently found in the more highly evolved soils of the coastal and inland valleys having finer textures and more highly developed colloidal properties than in the lighter, desert, and recently alluvial soils.

ACKNOWLEDGMENTS

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Effects of Reduced Sunlight on Water Stargrass (*Heteranthera dubia*)[1]

ROBERT E. WILKINSON[2]

WATER stargrass (*Heteranthera dubia* (Jacq.) Mac. M.) is a perennial, rooted, aquatic plant indigenous in the shallow lakes, ponds, and slow rivers of the eastern United States. It thrives in the drainage canals of northeastern Arkansas. This area is basically a reclaimed swamp with intermingled soils ranging from pure sand to heavy clay, and the water table is very close to the surface. Water stargrass reduces the carrying capacity of the drainage ditches, resulting in a rise in the soil water table and in flooded or waterlogged soils. Water stargrass makes its greatest growth in the spring during the season of heaviest load on the drainage canals.

During the summers of 1957 and 1958 the growth of water stargrass under natural conditions was very erratic. Because of the variable amounts of silt in the water and bands of trees along the banks of the drainage ditches it was suggested that reduced light might be one of the factors responsible for this erratic growth. Observations were made on the intensity of light at the bottom of two ditches. Honey Cypress Slough, carrying a heavy silt load, was measured at 1 percent of full sunlight at 18″ depth, and Buffalo Creek, carrying a lighter silt load, had 2 percent of full sunlight at 18″ depth.

Many authors have used aquatic plants for photosynthetic studies. The works of Arens (1), Gessner (2), Lowenhaupt (3), Manning et al. (4), Steemann-Nielsen (6), and Van der Veen (7) established many of the physiological effects produced upon aquatic plants by their environment and effects of aquatic plants on their environment. Meyer et al (5) found that water stargrass had an apparent photosynthetic compensation point between 1.8 and 0.68 percent of the sunlight available at the surface of the water. Since Meyer et al conducted only short-time experiments, the greenhouse experiment reported here was undertaken to measure the effect of shading on the growth of water stargrass over a longer period.

METHODS AND MATERIALS

Growth of water stargrass under 4 reduced light intensities was compared with growth in sunlight. The basipetal ends of twenty-five randomly selected 4- to 6- inch sections of water stargrass stems were imbedded about 2 inches in 2 kilograms of a fertile clay soil in each of forty 3-gallon aquaria. These sections rooted from the nodes within a few days. Each aquarium was filled with tap water.

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Twenty-four hours after implantation of the sections the aquaria designated for reduced light treatments were individually covered with shade cloth and white muslin to obtain the desired percentages of available sunlight: 23, 9.6, 5.8, and 2.8. Light intensity measurements were obtained at a water depth of 3 inches using a silicone solar battery attached to a shunted microammeter. Percentages of available sunlight in the shaded aquaria were calculated from the microamperes of current measured under each combination of shade cloth plus muslin and the current measured upon removal of the shading materials. Each treatment, including the control which received 100 percent available sunlight, was replicated 8 times within a single experiment and the experiment was repeated 6 times from April 1 to October 21, 1959. Total fresh weight of water stargrass per aquarium was recorded before imbedding in the soil. After a 4 weeks growth period the terminal total fresh and dry weights of water stargrass per aquarium were obtained. Fresh weights were measured to the nearest decigram after allowing the water to run off and blotting the sections with newspaper and dry weights were taken after the plants were dried to constant weight at 60±1° C.

The relative light quantity as influenced by season was obtained by measuring the area under the curve of a recording pyrheliometer[3] with a planimeter. The data represent only the area under the curve. However, this instrument records gram-calories/minute/square centimeter. Therefore, the area under the curve is proportional to the total energy available. In the final period of growth the pyrheliometer records ended on October 16. The total light available from September 23 to October 21 was estimated by multiplying the total light available from September 23 to October 16, inclusive, by 29/24.

RESULTS

The average fresh weight increase per aquarium and the average percentage increase over the six repetitions of the experiment are

Table 1. Fresh weight increase of 25 sections of water stargrass grown under varying percentages of natural sunlight during 4-weeks.[1]

Percent of full sunlight	Increase[2] in fresh weight		Percent of growth in full sunlight
	Grams/aquarium	Percent	
100	33.6	812	100
23	21.4	494	64
9.6	12.4	300	37
5.8	12.4	290	37
2.8	1.8	52	5

[1]Average total fresh weight per aquarium prior to testing=4.4 grams. Each value is the average of 48 measurements.
[2]LSD (1 percent level)=4.0 grams.

[3]Pyrheliometer records were graciously furnished by B. B. Bryan, Associate Agricultural Engineer, Department of Agricultural Engineering, University of Arkansas, Fayetteville, Arkansas.

shown in Table 1. Significant increases in fresh weight occurred at light levels as low as 5.8 percent of available sunlight (AS). The differences in growth at different light intensities were significant at the 5-percent level except between 9.6 and 5.8 percent AS. The data for 2.8 percent AS indicated that the compensation point for this plant over a long period was equal to or less than about 2.8 percent AS.

The effect of light intensity upon growth of water stargrass in each of the six repetitions of the experiment in shown in Figure 1.

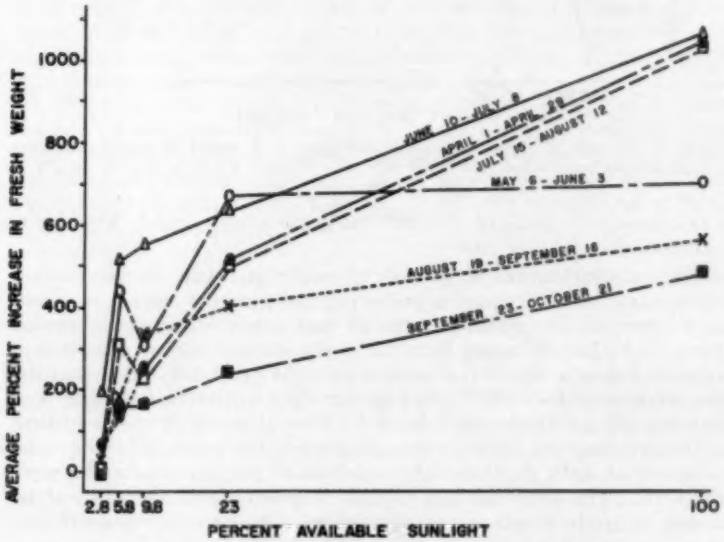

Figure 1. Percent increase in fresh-weight of water stargrass sections grown in varying relative light intensities during 6 different 4-week periods of the 1959 growing season.

The relations between the levels of growth of water stargrass and the relative light intensity available were similar in most respects in these repetitions. All curves show decreasing amounts of growth associated with decreasing light with a steep decline at lower light intensities. The average critical point of greatest decline was not determined between 23 and 5.8 percent AS. Growth at 2.8 percent AS was not significant except in one repetition. During the period June 10 to July 8 water stargrass made appreciable growth at this level of light intensity.

Since the point of greatest decline in Figure 1 was not constant, another presentation of the data (average total percent of growth vs percentage available light) was shown in Figure 2. This curve resembled a logarithmic curve of the type $Y = a + b \log_{10} \times$. Therefore a regression analysis was calculated using the average percent

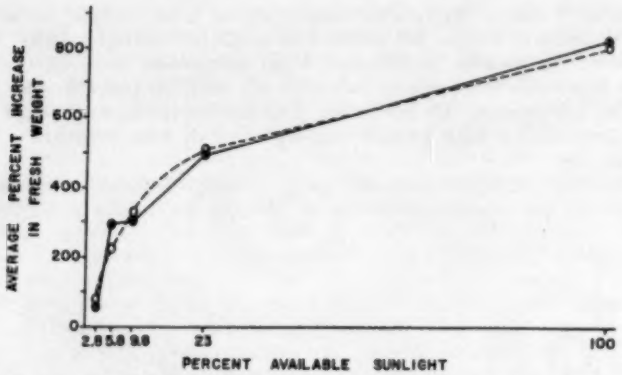

Figure 2. Percent of growth of water stargrass in 4 weeks at varying relative light intensities. Dotted line is the line of regression for $Y = a + b \log_{10} X$.

total growth vs $\log_{10}$ of the percentage available light. The correlation coefficient was +0.99.

The seasonal trend of growth of water stargrass in the various repetitions of the experiment during the growing season is shown in Figure 3. The greatest growth was made during the period June 10 to July 8. Since June 22 is the longest day of the year it appeared that a seasonal response to total available light quantity was likewise exhibited. Pyrheliometer data available for June 3 to October 16 are shown in Figure 3. They indicate that the growth of water stargrass throughout the season correspond to the total quantity of light available. An analysis of the correlation between the total light available per period of growth and the percent increase in fresh weight was undertaken. The correlation coefficient

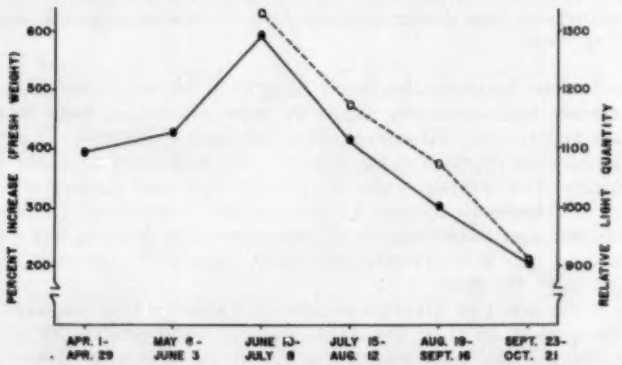

Figure 3. Total growth of water stargrass (solid line, scale left) and total light available (dash line, scale right) during the dates listed.

was 0.99 and it was concluded that a seasonal response of water stargrass to total available light was also exhibited.

DISCUSSION

Compared to growth of terrestrial plants in reduced light, 64 percent of the growth in full sunlight is a rather high value to have been obtained in 23 percent of full sunlight. The exact point of greatest decrease in rate of growth as related to light and other enviromental conditions needs further experimentation. It appears that this point changes with the season. Correlation of the average total growth and the logarithm of the percentage light available is unusual. Whether this correlation is a true representation of conditions in this and other aquatic plants or an anomaly of these data due to unrecognized factors is unknown.

Considering the minimum quantity of light needed for the production of sufficient fresh weight to be significantly different (5% level) from the material originally added to the aquaria, the following dates and percentages of available sunlight were found; 1. April 1 to 29—9.6 percent AS, 2. May 6 to June 3—5.8 percent AS, 3. June 10 to July 8—2.8 percent AS, 4. the following three periods—5.8 percent AS.

Quantitative data on the amount of light available to these plants at different depths below the water surface in natural streams are not available. Bartley and Otto[4] studied the quantity of light penetrating to various depths in 7 irrigation ditches in Colorado. These canals carry varying loads of suspended sediments. Bartley and Otto found an average reduction in light intensity of 80 to 90 percent of full sunlight at a water depth of 10 inches. In one canal they found a total light reduction of 95 to 100 percent within 5 inches of the surface. The maximum sunlight intensity (water surface) reported by these investigators was approximately 9300 foot-candles. Since the minimum amount of light in which significant growth of water stargrass was obtained was 2.8 percent full sunlight during the period June 10–July 8, assuming 2.8 percent full sunlight as necessary for growth under natural conditions these data indicate that a maximum of 300 foot-candles were required for the establishment and growth of water stargrass during that period. The minimum light requirements for the establishment and growth of water stargrass under natural conditions appear to be considerably lower than 300 foot-candles since this weed was growing in a ditch only 1 percent of the available sunlight at the waters' surface reached a depth of 18 inches.

SUMMARY

The correlation between the growth of water stargrass and the percentage total light available was tested in an experiment which

[4]Bartley, T. R., and N. E. Otto. Preliminary Field studies of submersed aquatic weeds in cooperation with the Northern Colorado Water Conservation District — 1959. Chemical Engineering Laboratory Report No. SI-26. U. S. Dept. of Interior, Bureau of Reclamation. Denver, Colorado. 1960.

was repeated 6 times over a 7-month period. The plants grown in 23, 9.6, 5.8 and 2.8 percent of the available sunlight produced 64, 37, 37, and 5 percent, respectively, as much growth in fresh weight as plants grown in unshaded aquaria. As an average of the repetitions of the experiment, the growth in fresh weight obtained in 2.8 percent of full sunlight was not significant. The growth in 2.8 percent of full sunlight was significantly greater (1% level) than O only during the period June 10–July 8. Plant growth in the different repetitions of the experiment during the 1959 growing season increased as the season progressed and attained a maximum during the period June 10–July 8. Growth decreased thereafter to the end of the experiment in October.

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Persistence of Soil-incorporated EPTC and Other Carbamates[1]

L. L. Danielson, W. A. Gentner, and L. L. Jansen[2]

The practical use of ethyl *N,N*–di–*n*–propylthiolcarbamate [EPTC] has been studied intensively by weed research workers since introduction of the herbicide in 1957 (3). Early studies were devoted to its evaluation as a soil-surface application for control of germinating weed seed. Studies conducted in 1958 showed that the activity of EPTC could be greatly increased by soil-incorporation immediately after application (4) and emphasis shifted to soil-incorporation research (1). In general, the accumulated applied research data on the use of EPTC in pre-planting, or lay-by, soil-incorporated treatments have emphasized the pressing need for basic investigations on the effects of soil composition and reaction, temperature, light, moisture, method and depth of incorporation, and method of formulation on the activity of this herbicide (6, 11, 12). A limited number of basic studies have been conducted on the effects of soil composition (5, 10, 13) and moisture (2, 9) on the performance and persistence of EPTC. However, basic research must be intensified if the excellent herbicidal potentialities of EPTC and other soil-active carbamates are to be utilized to their fullest extent.

The investigations on EPTC and other carbamates reported herein represent the initial phase of a program of studies on the basic factors affecting performance and persistence of soil-active herbicides.

Materials and Methods

Investigations on the persistence of soil-incorporated carbamates were conducted in the following phases: comparative field evaluations; edaphic and cultural factors; herbicide formulation factors; and field confirmation of laboratory and greenhouse studies.

A. *Persistence of several carbamates in preliminary field soil-incorporation studies.*

Initial field studies on soil-incorporated pre-planting carbamate herbicide treatments were begun in 1958 on a Delanco sandy loam with commercial formulations of the following carbamates: ethyl *N,N*–di–*n*–propylthiolcarbamate [EPTC]; 2–chlorallyl diethyldithiocarbamate [CDEC]; isopropyl *N*–(3–chlorophenyl) carbamate [CIPC]: *sec*–buytl *N*–(3–chlorophenyl) carbamate [BCPC]; 2–chloroethyl *N*–(3–chlorophenyl) carbamate [CEPC]; and isopropyl *N*–(3–methylphenyl) carbamate (referred to in this paper as MIPC). These carbamates were used in comparative field studies on the relation between herbicidal activity and persistence when the herbi-

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cides were soil-incorporated. The experimental area was plowed, disked, and smoothed with a Meeker harrow to prepare it for treatment. Whole plots for herbicide treatment were 12 × 100 feet, divided into three subplots 4 × 100 feet for planting dates. Subplot A received a pre-emergence (surface) treatment and subplots B and C received pre-planting (soil-incorporated) treatments at 2 and 4 weeks, respectively. A logarithmic sprayer (8) was used to treat the whole plots in a single application at a maximum rate of 16 lb/A of each herbicide in 33 gal of water on July 2, 1958. Treatments with EPTC were replicated three times and treatments with other herbicides were applied in single plots. Subplots B and C were double-disked to a depth of 3 to 4 in immediately after application and again 2 weeks later when subplot B was planted. Subplot C was disked a third time and planted 4 weeks after treatment. Plots were irrigated with 1 in of water immediately after incorporation and after each planting.

Air-temperature records were obtained with a thermograph. Accumulated temperature units in degree-hours above zero degrees Fahrenheit (°Hr F_0) were calculated by using planimeter readings from the thermograph charts.

Bio-assay plants used in these studies — mustard greens, snapbeans, annual ryegrass, lettuce, and cucumbers — were direct-seeded in single rows in each subplot. Results were determined on the basis of relative growth and visible injury ratings for each crop. Data are presented as phytotoxicity indexes.

B. *Effect of soil composition and method of irrigation on persistence of soil-incorporated EPTC in greenhouse studies.*

The field study described in A revealed that EPTC and CDEC were herbicidally active when soil-incorporated within the logarithmic rate-range used. EPTC was selected for continuation of soil-incorporation studies in the greenhouse during the winter of 1958. Emphasis was placed on the effect of method of irrigation and soil composition on persistence of the herbicide. The experimental design was a randomized split-split block involving three replications. Subplots for two soils and sub-subplots for top- and sub-irrigation were established.

A slightly acid potting soil (pH 6.2) containing 4.2 percent organic matter, and a 1:1 mixture by volume of this potting soil with washed pit sand were the two soils used in these studies. Metal flats 3.5 × 10 × 14 in were filled with approximately 16.3 lb of air-dry potting soil or 11.6 lb of the air-dry soil-sand mixture.

Treatments were made by spraying the flats of soil on an endless-belt experimental sprayer. A commercial formulation of EPTC (containing 6 lb of technical EPTC plus surfactants in sufficient kerosene to make a total volume of 1 gallon) was applied at rates of 1, 2, 4, and 8 lb/A in sufficient water to make a total of 40 gpa. The herbicide was incorporated by emptying the sprayed soil of each flat on wrapping paper and thoroughly mixing by a standard procedure before returning the soil to the flat.

The treated flats were placed in the greenhouse the day of treatment. A measured volume of annual ryegrass seed was planted in a single row across the shorter dimension of each flat. A specially prepared tool established a standard depth of ½ in for all plantings. Plantings were repeated at weekly intervals for 17 weeks. Top irrigations were made with a hose sprinkler as needed. The sub-irrigations were made weekly or as needed to provide optimum moisture conditions. Ryegrass in each planting was allowed to grow 4 weeks, and results were determined on the basis of number of plants and fresh and dry weights. Air- and soil-temperature data were recorded and integrated as described under A.

C. *Effect of organic solvent-carriers on persistence of soil-incorporated EPTC in greenhouse studies.*

The initial field and greenhouse investigations on the persistence of soil-incorporated commercial formulations of EPTC indicated the urgent need to determine the importance of kerosene, the organic solvent of the commercial formulation. Accordingly, greenhouse experiments were conducted on the persistence of soil-incorporated technical EPTC applied in kerosene and other organic solvents. A randomized-block experimental design, replicated three times, was employed. The experiment (Solvent Study I) was repeated (Solvent Study II).

The potting soil-pit sand mixture and metal flats described previously were used. Rates of 0, ⅛, ¼, ½, 1, and 2 lb/A active ingredient of the commercial formulation of EPTC were applied in water and equivalent rates of technical (98 percent pure) EPTC were applied in acetone, benzene, xylene, No. 2 fuel oil, and kerosene. In all instances 40 gpa of spray solution were used. Twenty selected oat seeds (var. Clinton) were planted ¾ inch deep immediately after soil incorporation as previously described and at weekly intervals for 6 additional weeks. Sub-irrigation was used throughout these experiments.

Each row of oats was harvested 4 weeks after its respective planting date, at which time the following data were recorded: (1) injury rating—0 to 10, where 0 equals no effect and 10 equals the death of the plant; (2) fresh weight-reduction percentage; and (3) height-reduction percentage. "Growth inhibition", as presented in Figure 2 was derived as follows:

$$\frac{(\textit{Injury score} \times 10) + \textit{ht. red. pct.} + \textit{wt. red. pct}}{3} = \text{Growth inhibition}$$

D. *Effect of surfactants on the persistence of soil-incorporated EPTC in greenhouse studies.*

Earlier studies demonstrated that the persistence of soil-incorporated EPTC depends partly on the organic solvent used as a carrier. Additional greenhouse studies were therefore conducted to determine whether the persistence of soil-incorporated EPTC is also influenced by surfactants.

A randomized-block experimental design replicated three times

was employed. Soil mixture, application rates of EPTC, total solution application rates, spray apparatus, and methods of incorporation in the soil were as described in C. The commercial formulation of EPTC was applied in water and in kerosene and compared with technical EPTC applied in a mixture of water and kerosene in the same proportions as found in the spray solutions prepared from the commercial preparation. Levels of 0.001, 0.01, and 0.1 percent (weight/volume) of three surfactants were used in combination with technical EPTC in a kerosene carrier. The surfactants used were S-145,[3] a nonionic polyoxyethylene sorbitan monolaurate (Tween 20); S-081, an anionic amine salt of alkyl aryl sulfonic acid (Emcol H-86C); and S-021, a cationic mono-alkyl polyoxyethylated amine (Ethomeen S/15).[4] The bio-assay plants, methods of seeding, and data recorded were as described in C.

E. *Effect of organic solvent-carriers on persistence of soil-incorporated EPTC in field studies.*

The greenhouse experiments showed that the persistence of soil-incorporated EPTC may be varied by employing different solvents. Therefore, field studies were conducted to compare one of the most persistent herbicide-carrier combinations found in the greenhouse studies with one of the least persistent combinations. These were respectively, the commercial formulation of EPTC in water and technical EPTC in kerosene,

A split-split-plot experimental design with four replications was used. Seed beds were prepared on a well-fertilized Delanco sandy loam by plowing, disking, and smoothing with a Meeker harrow. Each whole plot for treatment was 24 × 100 feet. Subplots for formulation were 12 × 100 feet.

Sub-sub-plots (A,B, C) for planting dates were 4 × 100 feet. Commercially formulated EPTC applied in water and technically pure EPTC applied in kerosene were used at a maximum rate of 8 lb/A with a logarithmic sprayer delivering 33 gpa of solution. Immediately after application the plots were disked to a depth of 3 to 4 in and four rows of oats were planted in sub-sub-plot A with a tractor-mounted gang planter. Overhead irrigation was used to maintain optimum soil moisture conditions for plant growth at all times. The sub-sub-plots B and C were disked and similarly planted 2 and 4 weeks, respectively, after herbicide treatment. Data were recorded 4 weeks after the planting dates and included determination of the highest herbicide rate for each planting date which caused no visible injury to the oats.

[3]S–numbers are inventory accession numbers, which will be used throughout this paper to refer to the respective surfactants.

[4]These surfactants were randomly selected from a large inventory of surface-active materials and are kerosene-compatible representatives of the various ionogenic classes. The mention of commercial products or companies anywhere in this paper does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over others of a similar nature not mentioned.

RESULTS AND DISCUSSION

A. *Persistence of several carbamates in preliminary field soil-incorporation studies.*

The preliminary field studies were conducted to evaluate the initial and residual herbicidal activity of six carbamates applied as pre-planting soil-incorporated treatments. A summary of the results (Table 1) shows that EPTC and CDEC were highly active as soil-incorporated treatments. The remaining herbicides were relatively inactive. The differential responses of the bio-assay plants to the herbicides are clearly shown. Ryegrass is a highly sensitive bio-assay plant for these herbicides. The data on the responses of the bio-assay plants to EPTC and CDEC (Tables 2 and 3) showed that EPTC was slightly more persistent than CDEC, based on the response of ryegrass. Effective rates of both of these herbicides persisted for more than 4 weeks.

Table 1. Summary of phototoxicity indexes of all herbicides as an average for all rates in each method of treatment for each crop in preliminary field soil-incorporation studies using the logarithmic sprayer. 1958.

Crop[a]	Treatment[b] method	Average phytotoxicity indexes[c] for all rates					
		EPTC	CDEC	CIPC	MIPC	BCPC	CEPC
Mustard greens	Pre-e	56	48	62	58	80	98
	Pre-pl 2 wk	49	78	86	109	58	80
	Pre-pl 4 wk	34	84	100	150	96	98
Snap-beans	Pre-e	85	97	72	78	91	94
	Pre-pl 2 wk	70	81	90	113	80	87
	Pre-pl 4 wk	75	80	92	138	96	96
Lettuce	Pre-e	27	65	44	79	98	100
	Pre-pl 2 wk	27	69	72	121	89	81
	Pre-pl 4 wk	26	77	90	142	114	91
Cucumbers	Pre-e	11	83	10	26	49	11
	Pre-pl 2 wk	6	59	71	101	113	93
	Pre-pl 4 wk	31	93	104	117	87	99
Ryegrass	Pre-e	0	0	0	104	40	0
	Pre-pl 2 wk	0	10	108	100	106	91
	Pre-pl 4 wk	4	23	93	115	108	94

[a]Mustard greens—*Brassica juncea* var. Southern Giant Curled.
Snapbeans—*Phaseolus vulgaris* var. Tendergreen.
Lettuce—*Lactuca sativa* var. Great Lakes.
Cucumbers—*Cucumis sativus* var. National Pickling.
Ryegrass—*Lolium multiflorum* var. Domestic Annual.
[b]Cumulative temperature record [c]Hr F_0/1000 for 2 wk=25.9, 4 wk=48.1.
[c]Average of height and injury ratings: 100=normal, 0=complete kill.

The extended period of persistence of soil-incorporated EPTC and the lack of persistence of similarly applied CIPC, previously reported (9), are in sharp contrast to the high herbicidal activity and limited period of persistence of both of these herbicides in pre-emergence applications. The studies reported herein emphasize the need for more intensive investigations on the effects of climatic factors, soil composition, and constituents of formulation on the performance and persistence of soil-incorporated herbicides.

Table 2. Effect of surface pre-emergence and soil-incorporated pre-planting applications of EPTC on several crops in preliminary field incorporation studies using the logarithmic sprayer. 1958.

Crop	Treatment method	Phytotoxicity indexes[a]					
		EPTC lb/A					
		0	1	2	4	8	16
Mustard greens	Pre-e	100	56	62	51	48	61
	Pre-pl 2 wk	100	68	57	40	47	31
	Pre-pl 4 wk	100	56	63	29	16	8
Snap-beans	Pre-e	100	89	87	85	82	84
	Pre-pl 2 wk	100	72	76	73	68	63
	Pre-pl 4 wk	100	104	88	78	62	43
Lettuce	Pre-e	100	34	38	41	11	11
	Pre-pl 2 wk	100	33	62	25	8	8
	Pre-pl 4 wk	100	57	40	17	9	9
Cucumbers	Pre-e	100	23	22	9	0	0
	Pre-pl 2 wk	100	15	8	3	3	0
	Pre-pl 2 wk	100	58	44	22	18	13
Ryegrass	Pre-e	100	0	0	0	0	0
	Pre-pl 2 wk	100	0	0	0	0	0
	Pre-pl 4 wk	100	9	8	5	0	0

[a]Average of height and injury ratings: 100=normal and 0=complete kill.

Table 3. Effect of surface pre-emergence and soil-incorporated pre-planting applications of CDEC on several crops in preliminary field incorporation studies using the logarithmic sprayer. 1958.

Crop	Treatment method	Phytotoxicity indexes[a]					
		CDEC lb/A					
		0	1	2	4	8	16
Mustard greens	Pre-e	100	97	56	50	0	38
	Pre-pl 2 wk	100	66	73	75	70	104
	Pre-pl 4 wk	100	85	87	85	82	82
Snap-beans	Pre-e	100	107	101	97	89	92
	Pre-pl 2 wk	100	75	66	77	82	106
	Pre-pl 4 wk	100	95	94	89	95	29
Lettuce	Pre-e	100	95	70	65	50	45
	Pre-pl 2 wk	100	96	67	52	38	91
	Pre-pl 4 wk	100	79	83	79	75	69
Cucumbers	Pre-e	100	103	79	81	70	84
	Pre-pl 2 wk	100	55	62	62	36	80
	Pre-pl 4 wk	100	94	91	99	96	87
Ryegrass	Pre-e	100	0	0	0	0	0
	Pre-pl 2 wk	100	50	0	0	0	0
	Pre-pl 4 wk	100	48	35	32	0	0

[a]Average of height and injury ratings: 100=normal and 0=complete kill.

B. *Effects of soil composition and method of irrigation on persistence of soil-incorporated EPTC.*

Striking differences in the persistence of pre-planting soil-incorporated applications of a commercial formulation of EPTC were evident between soils of low and high organic-matter content and between top- and sub-irrigated treatments (Figure 1). EPTC was more persistent in the soil of higher organic matter content. Top

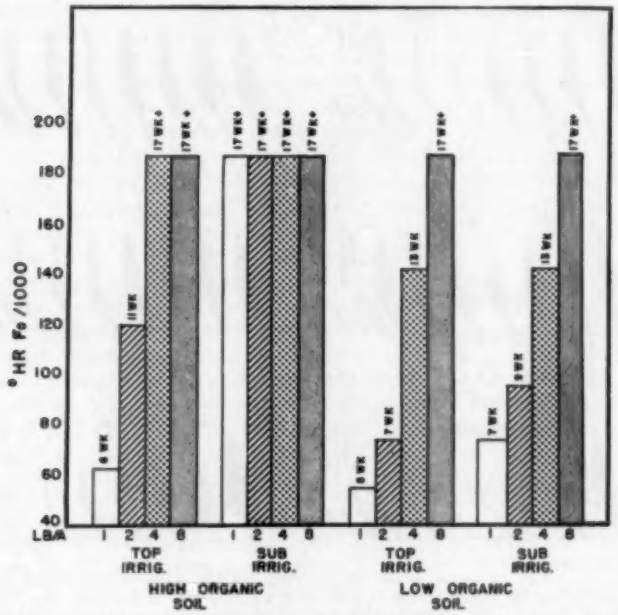

Figure 1. Duration of EPTC persistence as influenced by soil composition, method of irrigation, and rate of application. Duration in weeks of EPTC activity on ryegrass indicated by figure at top of each bar. Height of bars determined by accumulated temperatures during the persistence periods.

irrigation greatly hastened dissipation of EPTC in the heavy soil but did not materially change the rate of dissipation in the light soil. Differential aeration in the two methods of irrigation may have affected dissipation through oxidative mechanisms. Another possible explanation may be that physical movement of the chemical occurred

Obvious interactions between the methods of irrigation, soil composition, and rate of chemical application on persistence of soil-incorporated EPTC occurred. Whether persistence was a function of temperature or of time could not be ascertained under the conditions of this study.

C. *Effect of organic solvent-carriers on the persistence of soil-incorporated EPTC.*

In Solvent Study I the rates of dissipation of EPTC after soil-incorporation of the commercial formulation applied in water and technical EPTC applied in acetone, benzene, and xylene were slow (Figures 2 and 3). By contrast, soil-incorporated technical EPTC applied in No. 2 fuel oil and in kerosene dissipated rapidly. These data suggest the possibility of abbreviating or extending the period

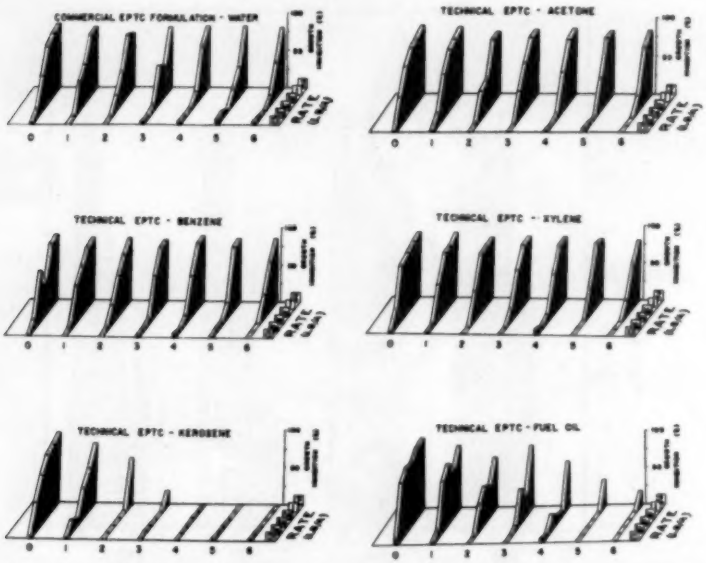
COMMERCIAL EPTC FORMULATION - WATER
TECHNICAL EPTC - ACETONE
TECHNICAL EPTC - BENZENE
TECHNICAL EPTC - XYLENE
TECHNICAL EPTC - KEROSENE
TECHNICAL EPTC - FUEL OIL

Figure 2. Effect of organic solvent carriers on the persistence of soil-incorporated EPTC on a soil of low organic-matter content. Activity of rates of application of EPTC on weekly plantings of oats shown as growth inhibition of 6 weekly periods. Time of bioassay in weeks following treatment date is shown on base lines.

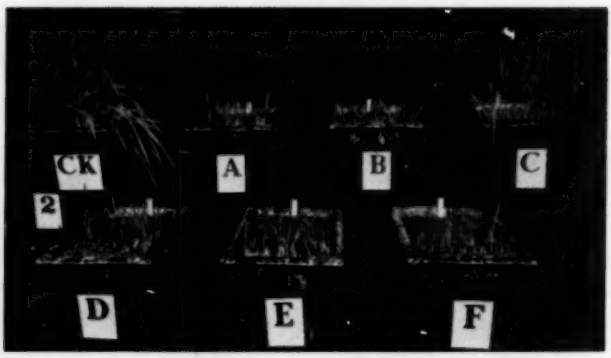

Figure 3. Representative flats showing comparative persistence of ¼ lb/A of EPTC applied in 6 solvents. Oat plantings, front to back, in each flat, made immediately, 1, and 2 weeks after chemical incorporation. A. Commercial formulation in water; B. Technical EPTC in acetone; C. Technical EPTC in kerosene; D. Technical EPTC in benzene; E. Technical EPTC in xylene; F. Technical EPTC in fuel oil.

of persistence of soil-incorporated EPTC by selecting the correct organic solvent-carrier. Results were confirmed in Solvent Study II.

The data indicate that fixation and/or chemical breakdown of EPTC occurred most rapidly when kerosene was used as the carrier. Samples of treated soil that no longer showed EPTC activity, on the basis of oat bio-assays, were extracted with acetone, and the resulting solutions were bio-assayed for EPTC by germinating cucumber seed in petri dishes as previously described for CIPC (7). Failure to detect any EPTC activity suggested that the EPTC either had been fixed so firmly on the soil that it was not released by the acetone or had been dissipated.

Further preliminary probing studies, in which the petri-dish cucumber bio-assay technique was employed to measure the herbicidal activity of EPTC, were conducted. EPTC was applied to heat-sterilized Delanco sandy loam, Codorus slit loam, and potting soil. Organic matter contents of these soils were low, intermediate, and high, respectively. The activity of a given rate of EPTC was found to be negatively correlated with organic matter content, indicating that physical fixation may influence EPTC persistence.

D. *Effects of surfactants on the persistence of soil-incorporated EPTC.*

Among common treatments the dissipation of EPTC in the Surfactant Study was more rapid than in either of the two solvent studies. The weight data for treatments involving the commercial formulation applied in water and technical EPTC in kerosene in Solvent Study I and the Surfactant Study were analyzed statistically. Weight differences found among plants of the fourth planting in the two experiments represented highly significant increases in the rate of EPTC dissipation in the Surfactant Study. These increases were restricted to the ½- and 1-lb/A rates of EPTC applied as the commercial formulation in water. Similar increased rates of dissipation were not detected among plants of the third planting or at other rates of EPTC application in either water or kerosene.

The weekly integrated temperature accumulations (°Hr F_0) during the first 4 weeks (Figure 4) show that air and soil temperatures were considerably higher in the Surfactant Study than in the solvent studies. Similarities of results in the two solvent studies correlate better with soil temperatures. The logical conclusion appears to be that soil temperature may influence the persistence of EPTC in certain solvents.

The commercial formulation of EPTC contains the herbicide dissolved in kerosene and sufficient surfactant to insure emulsification in water. The solvent studies demonstrated pronounced differences in persistence of commercial EPTC applied in water and technical EPTC in certain organic solvents. The Surfactant Study included solvent-formulation treatments in which one could differentiate possible effects of surfactants and of small amounts of organic solvent. Also included were a factorial series of three surfactants at three concentrations in the technical-kerosene system used pre-

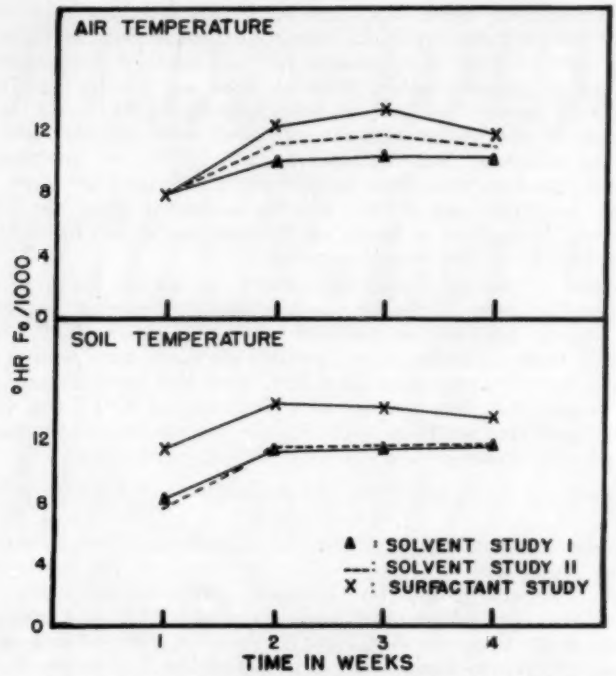

Figure 4. Integrated soil and air temperature during the first 4 weeks of three greenhouse experiments.

viously. In order to apply the water series containing technical EPTC and kerosene in the same proportions as in the commercial formulation, continuous and vigorous agitation was necessary.

Experimental differences due to surfactants were most pronounced in data of the third planting, 2 weeks after treatment. The weight data of this planting were subjected to statistical analysis.

A preliminary analysis of variance showed that all significant treatment differences were restricted to the three highest rates of EPTC application. At lower rates the herbicide had completely dissipated by the time of the third planting. Among the comparative treatments of the two EPTC formulations, i.e. commercial and technical, in the two solvent systems (Table 4), no differences due to formulation were evident in the water systems. In the kerosene solvent, dissipation of both formulations was greatly increased over the corresponding water treatments. Weight increases were significant for the commercial formulation and highly significant for the technical. At the 1-lb/A rate in kerosene the technical formulation of EPTC had completely dissipated but the commercial formulation had not. Under the conditions of this experiment,

Table 4. Effects of formulation-solvent mixtures on the residual activity of EPTC applied to a composted greenhouse soil mixture. Mean weights of plants grown on treated soil as percent of weights from untreated checks. Planting date: 2 weeks after EPTC applications.

EPTC formulation	Rate of EPTC lb/A	Solvent		Formulation means
		Water	Kerosene	
Commercial	½	84	128	
	1	0	18	41
	2	0	16	
Technical	½	81	130	
	1	0	121	62
	2	0	42	
Solvent means		28	76	

then, one may conclude that the surfactant component of the commercial formulation markedly increased the persistence of EPTC applied in a kerosene system. It did not affect persistence of EPTC applied in the water system.

Comparative evaluation of the three surfactants chosen for study in the technical EPTC-kerosene system demonstrated two principal effects of surfactants on the soil persistence of EPTC, namely, the individual surfactant and its concentration.

Comparative plotting of the weight data (Figure 5) and associated statistical comparisons showed that treatments containing the anionic surfactant (S-081) did not differ from those of the no-surfactant check treatments. In contrast, at the 1- and 2-lb/A rates of EPTC application, the nonionic (S-145) and cationic (S-021) surfactants increased the persistence of EPTC. Significant increases in persistence of both the 1- and 2-lb/A rates occurred at the 0.001- and 0.01-percent concentrations of the two surfactants. The highest surfactant concentration, 0.1 percent, increased the persistence of only the 1-lb/A rate of EPTC. Consequently, the total persistence of EPTC appears to be a complex function of amount of EPTC (rate), the solvent, the specific surfactant, and surfactant concentration.

E. *Effect of organic solvent-carriers on the persistence of soil-incorporated EPTC in field studies.*

Field studies comparing the persistence of soil-incorporated applications of a commercial formulation of EPTC applied in water and technical EPTC applied in kerosene confirmed the greenhouse studies showing that the EPTC-kerosene combination dissipated more rapidly. Amounts of commercial EPTC applied in water which were without effect on the bio-assay plants on subplots A (0-week planting time), B (2-week planting time), and C (4-week planting time) were 0.031, 0.217, and 0.440 lb/A, respectively. Corresponding amounts of technical EPTC in kerosene were 0.156, 0.299, and 2.000 lb/A. The accumulated air-temperature record showed 10.9 and 11.4 °Hr F_0/1000 for the first and second 2-week periods of record, respectively.

These demonstrations of pronounced differences in EPTC per-

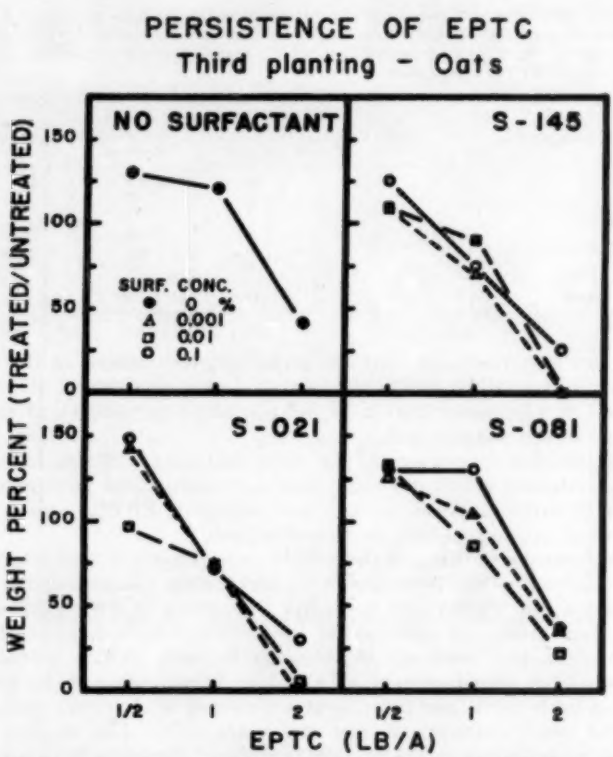

Figure 5. Response of oats to three rates of application of EPTC applied in kerosene containing three surfactants at three concentrations. Activity of EPTC-kerosene solutions alone (no surfactant) in upper left. Weight of plants grown on treated soils expressed as percent of weight of plants grown on untreated soil. Oats planted 2 weeks after EPTC applications.

sistence on relatively large field plots have two important implications. First, they substantiate the basic findings, obtained in greenhouse studies, that the persistence of EPTC is a function of several environmental and formulation factors. Second, they indicate the importance of persistence problems in the development of safe, effective methods of using soil-incorporated EPTC and other carbamates.

General Discussion

These investigations clearly demonstrate that soil-incorporated carbamates differ widely in selectively and persistence, as determined by performance. Extensive evaluation of experimental soil-incorporated carbamates in different solvent-surfactant systems may lead to greatly increased herbicidal efficiency of many old and new

chemicals belonging to this family. Many early evaluations of the carbamate series did not include soil-incorporation studies nor did they consider the effects of formulation factors on persistence of these chemicals.

SUMMARY

Studies were initiated in 1958 to determine the herbicidal activity of several soil-incorporated carbamate herbicides and to investigate the formulation, soil, and climatological factors involved in their persistence in the soil. Investigations included preliminary and final field studies and several greenhouse studies.

1. Field studies of EPTC, CDEC, CIPC, BCPC, CEPC and isopropyl *N*-(3-methylphenyl)carbamate indicated that EPTC and CDEC retained their herbicidal characteristics when soil-incorporated at a logarithmic rate-range of 1 to 16/A. EPTC was more persistent than CDEC, on the basis of bio-assay plant responses.

2. Ryegrass bio-assay tests in the greenhouse demonstrated that equivalent rates of EPTC persisted longer in soils of relatively high organic content and under conditions of sub-irrigation than in soils of relatively low organic content and under conditions of top-irrigation.

3. EPTC persistence was strongly influenced by the solvent-carrier system used in application. The most persistent systems were commercial EPTC in water and technical EPTC in acetone, benzene, and xylene. Technical EPTC in kerosene was the least persistent and in fuel oil, intermediate, The persistence characteristics of commercial EPTC in water and technical EPTC in kerosene were confirmed in field studies.

4. High soil temperatures appear to increase the rate of dissipation of commercial EPTC applied in water on a soil relatively high in organic matter. Temperature effects on rate of dissipation could not be detected when technical EPTC was applied in kerosene.

5. EPTC persistence was affected by the addition of specific surfactants and surfactant concentrations. Surfactant action, when present, resulted in increased persistence of EPTC in a kerosene solvent system.

6. The implications of these results to future research and development of soil-incorporated herbicides are discussed.

ACKNOWLEDGEMENTS

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Dissipation of Simazine from the Soil[1]

O. C. Burnside, E. L. Schmidt, and R. Behrens[2]

Simazine, 2-chloro-4, 6-bis (ethylamino)-*s*-triazine, can give excellent control of annual weeds in corn for most of the growing season. However, a number of workers have reported injury to susceptible crops the year following applications of four or five pounds of simazine per acre (4, 8, 10, 14). The persistence of simazine is thus of considerable importance to the user who practices crop rotation. The purpose of this study was to investigate factors involved in the dissipation of simazine from the soil.

Methods and Materials

Both technical and formulated simazine (Simazine 50W)[3] were used in these experiments. The soybean (*Glycine max* (L.) Merr. var. Flambeau) bioassay procedure, used for all bioassays herein, has been reported in detail previously (1). The desired amount of simazine was mixed with 500 g of Waukegan silt loam soil and placed in clay pots. Six soybean plants were grown in each pot for 30 days following planting, after which the above-ground parts of the plants were harvested, dried, and weighed. Reductions in plant weight were proportional to simazine concentration in the soil. Most bioassays were conducted in a growth chamber where the temperature was about 73° F. during an 18 hour exposure to 800 footcandle light intensity, and 68° F. during the 6 dark hours of each 24 hour cycle. A few bioassays were completed in a greenhouse at a temperature of about 75° F. Plant materials harvested were dried at 212° F. for three days before weighing. All soil weights were calculated on an oven-dry basis. The analyses of the soils used by procedures reported previously (1) are given in Table 1. A randomized block experimental design was used throughout the study.

Leaching of simazine was carried out in the field, and the residual simazine at different soil depths was determined by bioassay. Each 40 by 45 inch field plot was surrounded by a 6-inch metal strip driven 4 inches into the soil. The simazine was applied in 80 gpa of water with a small plot sprayer designed by Robinson and Dunham (7). Water (0, 1, and 2 inches) was applied with a sprinkling can in ½-inch increments with a one-hour interval

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[3]Supplied through the courtesy of the Geigy Chemical Corporation, Yonkers, New York.

Table 1. Analyses of the soils used in this study.

Soil type	pH	Organic matter %	Cation exchange capacity*	Sand % 2–0.05 mm	Silt % 0.05–0.002 mm
Waukegan silt loam	5.4	4.0	16.8	19.4	52.0
Waukegan silt loam†	5.5	2.9	13.6	19.6	54.8
	Clay % below 0.002 mm	Available P lb/A	Exchangeable K lb/A	Moisture % at 1/3 atm.	Moisture % at 15 atm.
Waukegan silt loam	28.6	180	480	25.2	9.6
Waukegan silt loam†	25.6	—	—	22.2	7.7

*Cation exchange capacity is expressed in millequivalents per 100 grams of oven-dry soil.
†This soil was treated with 12 pounds of simazine per acre one and two years prior to the collection date.

between applications. After the water was applied rainfall was excluded for 7 days with a plastic cover, and then samples of 500 gm each were taken at the 0-½-, ½-1-, 1-3, and 3-6-inch soil depths.

Bioassays were used in an attempt to study the uptake of simazine by plants. A Waukegan silt loam soil was treated with 0, 1, 2, 4 and 8 ppmw of simazine in the greenhouse. Soybeans were grown for 30 days, then their top growth was removed, dried, weighed, and discarded. After an 11 or 12-day period the soil was thoroughly mixed, returned to the same pot, and another planting of soybeans was grown in this manner.

The deactivation of simazine under various temperature and pH conditions was studied by subjecting flasks containing 300 ml of 200 ppmw of simazine suspensions (buffered at pH 4, 6, and 8 with McIlvaine's standard buffer solutions) to 68, 104, 176, 212, and 248° F. for 24 hours. A water bath was used to maintain temperatures of 68, 104, 140, and 176° F. and an autoclave was used to obtain 212 and 248° F. Following these exposures five ml aliquots of these suspensions were mixed with Waukegan silt loam soil and bioassayed to determine the degree of deactivation.

The possible loss of simazine phototoxicity by volatilization was studied. Simazine suspensions, buffered at pH 4 and pH 8, were placed in a boiling water bath for 24 hours. Vapors given off the suspensions were passed through a water trap and a column containing 100 grams of Waukegan silt loam soil to absorb any simazine in the vapors. A stream of air passing through this apparatus carried the vapors through the soil column. The soil in each column was then mixed with an additional 400 gm of Waukegan silt loam and bioassayed to detect simazine adsorbed from the vapors. Samples of an unheated simazine suspension, the simazine suspension that had been heated, and the condensate in the water traps of each simazine suspension at pH 4 were added to soil and bioassayed also.

The influence of simazine on total microbial activity was measured by determining carbon dioxide evolution of 100 gm samples of Waukegan silt loam soil treated with 0, 8, 64, or 4096 ppmw

of technical simazine. The soil was moistened to field capacity and incubated for 30 days at 85° F. while attached to a carbon dioxide absorption apparatus (9).

The influence of simazine on nitrification in soil was studied using Waukegan silt loam soil leached free of nitrates. Three replications of 0, 8, 64, and 4096 ppmw of formulated simazine were mixed with 50 gram soil samples. The amount of nitrate produced during 45 days incubation at field capacity and 86° F. was measured by the phenoldisulfonic acid procedure (5).

The role of microorganisms in the inactivation of simazine was studied by comparing the persistence of simazine in sterile and non-sterile Waukegan silt loam soil. Soil was sterilized in two-quart jars by autoclaving at 253° F. for three hours. This treatment was repeated after a two-week interval. Simazine sterilized separately in methyl alcohol was added at the rate of 4 ppmw to the sterilized and non-sterilized soil. Beginning in September, 1958, and at 30-day intervals following, sterile and non-sterile soils were bioassayed for residual simazine. Preliminary investigations indicated that sterilizing simazine with methyl alcohol and autoclaving soil to be used did not influence the results of the bioassay.

Soil microorganisms capable of degrading simazine were sought by plating a simazine-treated soil sample that had been incubated at field capacity for six months at 85° F. The medium used consisted of 0.2 gm of K_2HPO_4, 0.1 gm of KH_2PO_4, 0.1 gm of $MgSO_4$, 0.005 gm of $FeSO_4$, 15 gm of agar, and two drops of polyethylene sorbitan monooleate (Tween 80) per liter of deionized water. After sterilizing the medium, methyl alcohol sterilized simazine was added aseptically to give a 400 ppmw simazine suspension.

Five isolates that survived repeated transfers of this medium were selected to inoculate a liquid medium of the mineral composition listed above plus 1.5 gm ammonium sulfate and two drops of Tween 80 per liter. After 15 days and again at the end of 30 days of shaken incubation, duplicate aliquots from each culture treatment were bioassayed.

RESULTS AND DISCUSSION

Leaching of simazine in soil.

There was a significantly greater concentration of simazine in the 0-½- and ½-1-inch than in the 1-3 and 3-6-inch soil depths. When 1 inch of water was applied following an application of 2 pounds of simazine per acre, 4, 2, 1, and 0 ppmw of simazine were found with bioassays at the 0-½- ½-1-, 1-3-, and 3-6-inch soil depths, respectively. When two inches of water were applied, 4, 4, 1, and 1 ppmw of simazine were detected at the 0-½-, ½-1-, 1-3-, and 3-6-inch soil depths, respectively. Doubling the herbicide rate resulted in an increase in the amount of simazine leached into the ½-1-inch depth by the 1-inch application of water. Sheets (11) has reported similar results on the leaching of simazine. While the rate of

leaching was not rapid, it would be a contributing factor in the dispersion of simazine in the soil.

Plant uptake of simazine.

Table 2 illustrates the reduction of simazine toxicity when successive plantings of soybeans were grown on the same soil. When the third crop was grown the 1 ppmw simazine concentration no longer inhibited soybean growth. Decreased growth inhibition was

Table 2. Top growth yields of soybeans, expressed as percentage of the check, when three successive crops were grown on simazine-treated Waukegan silt loam soil.

Thirty-day cropping period	Soybean yields, percentage of check				
	Simazine ppmw				
	0	1	2	4	8
1st crop	100	75	45	20	16
2nd crop	100	94	72	35	29
3rd crop	100	100	87	51	27

evident with each successive 30-day crop grown on soil treated with 2 and 4 ppmw of simazine, also. This trend was less pronounced when 8 ppmw of simazine was used. Laboratory studies show no microbial breakdown of simazine during the first four months of incubation in soil. Also, Davis et al. (2,3) using radioisotope techniques obtained evidence of plant uptake of simazine, and showed that uptake by plants continued for a considerable period of time. On the basis of such results, it appears that the reduced phytotoxicity after repeated croppings, observed in this study, is due to plant uptake of simazine since the soybean plants were discarded after each 30-day cropping period.

Influence of temperature and pH on simazine phytotoxicity.

A detoxification of simazine was noted in attempts to sterilize media containing simazine by autoclaving. This indicated the possibility of loss of simazine phytotoxicity from the soil at higher soil temperatures. High temperatures (212 and 248° F.) and low pH (pH 4) partially detoxified simazine in water suspensions. Bioassays indicated significant losses of 25 to 75 percent of the simazine at aforementioned temperatures and pH levels (Table 3). Temperature appeared to be the most important factor though pH had an effect, since simazine suspensions buffered at pH 4 were detoxified to a greater extent than were suspensions buffered at pH 6 and 8. Tests in this study indicated that there is little likelihood of the loss of simazine phytotoxicity from soil at normal soil temperatures.

Volatilization of simazine.

The reason for the loss of phytotoxicity in the previous experiment could not be determined in that test. However, results of this experiment indicate that the loss of phytotoxicity of simazine

Table 3. Simazine concentrations remaining in water suspensions after a 24-hour exposure to various pH and temperature levels. An aliquot of the original suspension equivalent to 2 ppmw of simazine was bioassayed.

Temperature in °F.	Simazine, ppmw		
	pH 4	pH 6	pH 8
68	2 a*	2 a	2 a
104	2 a	2 a	2 a
140	2 a	2 a	2 a
176	2 a	2 a	2 a
212	1.5	2 a	2 a
248	0.5	1 b	1 b

*Numbers followed by the same letter do not differ significantly at the 5 per cent level using Duncan's multiple range tests (13).

in suspension, exposed to high temperatures and low pH, may be due to deactivation rather than volatilization. Soybeans grown in soil exposed to vapors arising from boiling simazine suspensions were not inhibited, even though a considerable amount of the simazine was detoxified (Table 4). Similarly, bioassays indicated essentially no simazine in 20 ml of condensate given off by boiling simazine suspensions. On the other hand, Davis et al. (2) found that 50 percent of the simazine placed on a metal surface and subjected

Table 4. Phytotoxicity of heated simazine suspensions and vapors from heated suspensions as indicated by bioassay.

Material used in bioassay	pH of simazine suspension	Simazine ppmw
5 ml sample of unheated suspension	4	4.0 a*
5 ml sample of heated suspension	4	1.9 b
Soil exposed to vapors from heated suspension	4	0.1 c
Water exposed to vapors from heated suspension	4	0.2 c
5 ml sample of unheated suspension	8	3.6 a
5 ml sample of heated suspension	8	2.6 ab
Soil exposed to vapors from heated suspension	8	0.0 c

*Numbers followed by the same letter do not differ significantly at the 5 per cent level using Duncan's multiple range tests.

to 160 to 165° F. for a two-month period volatilized. Smaller percentages of simazine volatilized at lower temperatures, less volatilized from sand than metal, and even less volatilized from clay than sand. However, it seems that volatilization would not be a major factor in the loss of simazine from soil at temperatures normally found in the field.

Influence of simazine on microbial activity.

Carbon dioxide evolution over a 30-day period was utilized to determine whether simazine influenced the over-all activity of soil microorganisms. Simazine had no significant effect on carbon dioxide evolution from soil samples even though application rates up to 4096 ppmw were used (Table 5). This indicated that microbial activity in soils was not markedly inhibited or stimulated by simazine. The possibility existed that microorganisms might require more than a 30-day period to adapt to a simazine substrate. There-

Table 5. Carbon dioxide evolution from 100 grams of Waukegan silt loam soil treated with various rates of technical simazine.

Simazine ppmw	Milligrams of carbon evolved as carbon dioxide in a 30-day period	
	No prior simazine treatment	Prior simazine treatment*
0	32.3 a**	38.9 b
8	33.1 a	38.2 b
64	32.3 a	40.0 b
4096	31.3 a	36.0 b

*This soil was treated with 12 pounds of simazine per acre one and two years prior to the collection date. Also, it contained 1.1 per cent more organic matter than the soil which received no prior simazine treatment (Table 1).

**Numbers followed by the same letter do not differ significantly at the 5 per cent level using Duncan's multiple range tests.

fore, soil was collected from field plots treated with 12 pounds of simazine per acre one and two years prior to the collection date. Again simazine treatments had no appreciable effect on carbon dioxide evolution (Table 5).

Influence of simazine on nitrate formation.

Whereas carbon dioxide evolution is an index of the activity of the general soil microflora, the formation of nitrate reflects the activity of the specific nitrifying segment of the population. The nitrifying microflora is usually considered (12,6) to be more sensitive to herbicides than is the general soil population. Nitrate formation in the Waukegan silt loam soil, however, was not impaired even by large applications of simazine. The control soil formed 43.2 micrograms of nitrate nitrogen per gram of soil, while simazine treatments at 8, 64, and 4096 ppmw resulted in 35.1, 39.4, and 45.9 micrograms per gm, respectively. Therefore, it appears that simazine has little influence on the soil microflora.

Microbial deactivation of simazine in soil.

Deactivation of simazine occurred in non-sterile Waukegan silt loam soil incubated in the greenhouse with most of the deactivation occurring after the fourth month (Table 6). This might mean an adaption period was required by microorganisms involved. Sterile soil was available for only the first six bioassays because of contamination losses. Simazine in soil incubated outside was not deactivated from October through April, when temperatures were low, but from May through July, when temperatures were higher, the rate of simazine deactivation became quite rapid. It seems evident on the basis of the sterile versus non-sterile soil and the greenhouse versus outside incubation (i.e., temperature differential) comparisons that soil microorganisms do deactivate simazine. However, simazine is less available as a substrate in soil than many herbicides and its inactivation by soil microorganisms is relatively slow.

Simazine inactivation by cultures isolated from soil.

The five isolates obtained from soil that had been treated with simazine included 2 fungi, *Penicillium purpurogenum* (series) Stoll.,

Table 6. Simazine concentrations, as determined by bioassays, following incorporation of 4 ppmw in sterile and non-sterile Waukegan silt loam at field capacity for varying periods.

30-day incubation periods	Simazine, ppmw		
	Sterile soil 85° F.	Non-sterile soil 85° F.	Non-sterile soil outdoors
Beginning	4 a*	4 b	4 e
October	4 a	3 b	4 e
November	4 a	4 b	4 e
December	4 a	4 b	4 e
January	4 a	3 b	4 e
February	4 a	1.7 c	4 e
March	—	1.4 c	4 e
April	—	1.2 c	4 e
May	—	0.6 d	1.1 f
June	—	0.7 d	1 f
July	—	0.4 d	0.6

*Numbers followed by the same letter do not differ significantly at the 5 per cent level using Duncan's multiple range tests.

and *Aspergillus ustus* (group) (Bain.) Thom and Church, and 3 actinomycetes, all identified as *Streptomyces* sp. Inoculation of these cultures into liquid medium with simazine as the carbon source and ammonium sulfate as the nitrogen source gave the results listed in Table 7. Although each of the cultures survived five serial transfers in simazine medium and grew moderately well in simazine solution, it is clear that none inactivated the simazine during the 30-day incubation period. It is likely that the demands for cell synthesis were too low to result in measurable inactivation of simazine within the test period.

Table 7. Concentrations of simazine in ppmw remaining in solution cultures after incubation at 77° F. in the presence of each of five microorganisms.

Microorganism	Incubation period on the shaker	
	15 days	30 days
Penicillium purpurogenum (series) Stoll	4.0 a*	3.9 a
Aspergillus ustus (group) (Bain.) Thom and Church	4.0 a	4.0 a
Streptomyces sp.	4.0 a	3.9 a
Streptomyces sp.	3.9 a	4.0 a
None	4.0 a	4.0 a
None (not shaken)	4.0 a	4.0 a

*Numbers followed by the same letter do not differ significantly at the 5 per cent level using Duncan's multiple range tests.

SUMMARY

Methods whereby simazine may be dissipated from the soil were studied.

Simazine was leached into Waukegan silt loam by percolating water, indicating this could be a factor in simazine dispersion into the soil. The depth to which simazine moved was not great but it increased with the amount of water applied.

Repeated croppings of soybeans in simazine-containing soil resulted in reduced phytotoxicity.

High temperatures (212° and 248° F.) and to a lesser extent low pH (pH 4) caused a significant deactivation of simazine in water suspensions. However, the very high temperatures necessary for this deactivation indicate that temperature would not be a significant factor in simazine deactivation in the field. Loss of simazine by volatilization was not detectable even when simazine suspensions were boiled.

Applications of simazine used for selective weed control or even soil sterilization did not adversely affect the nitrification process or the over-all microbial activity in Waukegan silt loam soil.

Deactivation of simazine occurred under conditions conducive to microorganism growth, but the rate of breakdown was fairly slow. The phytotoxicity of 4 ppmw of simazine in non-sterile Waukegan silt loam soil at field capacity was reduced 90 per cent during 10 months of incubation at 85° F. No deactivation of simazine occurred in sterile or frozen soil.

Five microorganisms that were able to subsist for three months in media containing simazine as the sole source of nitrogen and nearly sole source of carbon were isolated and identified. However, these microorganisms did not deactivate simazine in solution cultures during a 30-day incubation period.

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Studies in the Life History of *Artemisia absinthium*[1]

G. W. Selleck[2] and R. T. Coupland[3]

Absinth wormwood *(Artemisia absinthium L.)*, a perennial, native to Europe (3), has become abundant during the last decade in several locations in western Canada. Reports received during the Saskatchewan Weed Survey[4] suggest that at least some of the introductions into this area were by immigrants from Europe, who believe it to have medicinal properties. Silver-grey in color and covered with fine hairs, the herbaceous stem grows to a height of 2 to 4 ft, often branching profusely. The leaves are up to 5 in. long and are two to three times divided into lanceolate segments. Numerous, drooping heads, about 3/16 in. in diameter, develop in leafy panicles. Yellow or purple florets are borne on a hairy base. The whole plant has a strong, penetrating odor, common in other species of this genus. In Europe it is used as a stimulant (tea and liquor) (1).

The first report of the weedy nature of this species in western Canada resulted from the discovery (by S. Zilke) in 1954 of serious infestations along fences and roadsides, in pastures, and in farmyards in the Luseland-Denzil and Kealy Springs areas of Saskatchewan (2). In 1956 extensive infestations were found in the Penzance-Dilke region of Saskatchewan and in parts of Manitoba (2). Small infestations have since been located by the authors in a score of locations throughout Saskatchewan (one being beyond the northern edge of agricultural settlement), and the weed has been observed in Alberta and the Dakotas. One of the infestations in Manitoba occurs along roadsides for 65 miles, while in each of two Saskatchewan regions the weed has spread throughout an area approaching 100 square miles. While the most dense and extensive stands occupy pastures, cultivated fields are frequently invaded from the vigorous stands along roadsides and fence lines (Figure 1).

In addition to reducing pasture and crop yields, absinth is considered of economic significance because consumption by cattle has resulted in tainted dairy products and grain from infested fields has been rejected for commercial sale because of the danger of tainting flour. The objectionable odor has caused illness of persons working in infested fields (2).

The purpose of this paper is to report on some aspects of the behavior of the species under representative environments in Saskatchewan, where life history studies have been conducted during four growing seasons.

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[4]Coupland, R. T., et al. Survey of the abundance and distribution of persistent perennial weeds in Saskatchewan. Unpubl. report. 1955.

Figure 1. Absinth wormwood infesting a roadside near Denzil, Saskatchewan (August, 1958).

METHODS AND PROCEDURES

Studies were initiated in 1956 habitats in three regions within the dark-brown soil zone, in which the texture of surface soil ranged from loam to clay loam and land use varied from cultivated land to native pasture. Information concerning phenology and development was obtained by detailed observation. Vigor and competitive ability were measured annually in terms of rate of spread along 16 marked, 0.5 m wide belt transects (nearly two miles in total length) and changes in density within 638 permanent 0.5 sq m quadrats along these transects. Infestations were considered to be continuous when gaps between plants in the 0.5 m belt did not exceed 3 ft.

The study began in the last year of a moist period (1950-56) in which annual precipitation in this region averaged 20 percent above the long-term mean (15 in.), but the last three years were drier than average by approximately 15 percent.

RESULTS

Establishment and growth.

Natural colonization occurs entirely from seed. The seed germinates from mid-summer until autumn, and a rosette develops. The rosette (shoot) resumes growth in the early spring, reaching a height of 1 to 2 in. by May 1 and 8 to 10 in. by June 1. One or more flowerstalks are produced by each vigorous shoot about mid-July and seed is mature by late August. The crown of an established plant is as large as 4 in. in diameter, with several shoots occupying an area of soil surface up to 8 in. in diameter. Such a plant has as many as 23 flowerstalks ranging from 1 to 5 ft high and shading an area 4 ft in diameter. Although roots produce shallow lateral branches from below the crown, there is no evidence of vegetative reproduction from them. Nevertheless, infestations in

cultivated fields are sometimes extended by transport of cuttings of underground parts by tillage implements.

The tap root occasionally reaches 2 in. in diameter, with lateral branches extending 6 ft in all directions. Tillage with a moldboard plow sometimes fails to sever such a well-developed, tough root system.

Disturbed areas with lack of competition from other plants provide conditions which are most favorable for germination and establishment of seedlings. Among the situations where they are most commonly found are bare soil in overgrazed pastures, in soil disturbed by rodents, where native grassland has been disturbed by piles of manure, soil or hay, in sparse stands of seeded crops of perennial grass or grain, in land abandoned from cultivation, and in farmyards where trampling and other disturbances have disrupted the native plant cover.

The occurrence of seedlings among established plants of other species has been observed. They occasionally occur in grain crops in autumn, and are most abundant where the crop is thin. The weed was found at one site to be growing vigorously within a plantation of trees which provided an estimated 60 percent crown cover. While seedlings were found in a stand of ungrazed uplant native grass *(Stipa comata)*, they did not survive this high degree of competition. In ravines, however, absinth seedlings survived in relatively moist positions and infestations advanced by means of seed which migrated in flowing water.

Rate of spread.

Annual changes in rate of spread are expressed as a percentage of each 0.5 m belt transect which was infested with the weed (Table 1). These data illustrate the ability of the plant to invade areas which have been abandoned from cultivation, even when seeded to a vigorous forage grass. More surprising, however, is its abundance in native pasture lands, presumably as a result of disturbance associated mainly with grazing.

During the study period the weed continued to advance in the abandoned and regrassed areas, but receded in native pastures. At the most northwesterly site, however, absinth infestations increased in all environments. These trends were interrupted temporarily in some sites by fluctuations in the opposite direction, presumably in response to weather conditions.

The recessions in native grassland were the result of death of both seedlings and established plants in relatively xeric positions during the dry weather of 1957 to 1959. This was particularly evident on dry knolls, where the effects of drought were intensified by rolling topography. At one site, accumulations of salt at the soil surface caused the death of absinth along the margin of a slough, even though soil moisture was abundant. Drier conditions also resulted in a decrease in the vigor of upland plants. While flowering stems 1 to 2 ft high were abundant in 1956, by 1959 only vegetative shoots 2 to 3 in. high were produced. In adjacent, moist

Table 1. Percentage of soil surface infested with *Artemisia absinthium*, 1956–1959.

Location	Site no.	Habitat	Length of transect ft.	Percentage of transect infested				Per cent change 1956–1959
				1956	1957	1958	1959	
Denzil	1	Moderately grazed native pasture	732	30	20	25	32	+7
Dilke	5	Moderately grazed native pasture	680	28	21	14	10	−65
Dilke	6	Moderately grazed native pasture	715	26	22	17	18	−32
Markinch	7	Overgrazed native pasture	700	48	51	39	43	−10
Kealy Springs	9	Moderately grazed native pasture	179	54	58	40	50	−6
Denzil	3	Abandoned regrassed	18	89	89	89	90	+1
Kealy Springs	9	Abandoned regrassed	712	86	84	56	81	−6
Kealy Springs	9	Abandoned annual weed stage	15	63	100	100	100	+74
Denzil	1	Brome grass, seeded 1954	1010	37	32	70	77	+109
Denzil	2	Brome grass, over-grazed	877	64	43	94	—	+47
Denzil	4	Brome grass, over-grazed	1558	35	48	80	91	+159
Denzil	1	Brome grass, old stand	1039	2	2	24	51	+2734
Kealy Springs	9	Crested wheat grass	264	60	59	40	47	−22
Denzil	1	Cultivated	119	85	27	60	13	−84
Denzil	3	Cultivated	620	2	0	2	0	−100
Kealy Springs	8	Cultivated	633	9	50	2	2	−81

ravines, plants continued to grow and spread vigorously in competition with western snowberry *(Symphoricarpus occidentalis)*.

Moderate cultivation was effective in reducing the extent of the infestations (up to 80 percent) of established plants.

Density of stands.

Density is expressed in Table 2 as the average number of shoots per 0.5 sq m. Examination of these data reveals that dense stands occur in competition with both native and seeded grasses. Increases

Table 2. The number of shoots of *Artemisia absinthium* per 0.5 m² in various habitats, 1956–1959.

Location	Site no.	Habitat	No. of quadrats	Shoots per 0.5 m²				Per cent change 1956–1959
				1956	1957	1958	1959	
Denzil	1	Moderately grazed native pasture	8	29	10	13	13	−53
Dilke	5	Moderately grazed native pasture	8	50	27	32	19	−63
Dilke	6	Moderately grazed native pasture	10	26	17	26	12	−55
Markinch	7	Moderately grazed native pasture	16	28	38	29	23	−17
Kealy Springs	9	Moderately grazed native pasture	46	18	16	4	7	−62
Kealy Springs	9	Abandoned naturally regrassed	73	29	27	15	14	−51
Kealy Springs	9	Abandoned annual weed stage	5	12	23	22	10	−16
Denzil	1	Brome grass, seeded 1954	10	24	24	54	68	+191
Denzil	2	Brome grass, overgrazed	18	26	15	19	—	−24
Denzil	4	Brome grass, overgrazed	13	18	38	20	34	+88
Kealy Springs	9	Crested wheat grass	69	7	7	2	2	−68
Denzil	1	Cultivated	2	11	4	7	2	−77
Denzil	3	Cultivated	190	tr.	0	tr.	0	−100
Kealy Springs	8	Cultivated	170	tr.	2	tr.	tr.	−75

in density occurred in two of three locations at the most northwesterly site (Denzil) in competition with introduced grasses. Densities decreased, however, at all of the other sites. During the study period there was a tendency for densities to decrease proportionately more than associated decreases in area occupied. Where the weed increased in area, there was no consistent increase in density.

DISCUSSION

Absinth apparently needs a relatively abundant supply of moisture in order to thrive. Its sudden appearance as a weed in the area was probably associated with the above-average moisture conditions which prevailed during 1950 to 1956. It was not able to maintain this level of abundance during the dry weather of 1957 to 1959, except at Denzil. The response of this species to climate may also be significant. Extensive infestations known to occur in Manitoba exist under a more humid climate than that of the present study.

After establishment, the plant is able to withstand vigorous competition. In Europe, this ability has been related to the presence in the leaves of an alkaloid which has a depressing effect on neighboring plants of other species (4).

If absinth succumbs on the upland during a succession of drought years, it will probably be able to survive in the moist habitats of depressions and sheltered slopes, thus maintaining itself for reinvasions during moister weather cycles. The extent of these invasions will be related to the availability of disturbed soil for establishment of seedlings.

In Saskatchewan, infestations of absinth have been controlled in various ways. Fall tillage results in elimination of seedlings, but intensive tillage during the subsequent year is necessary to kill older plants. Vigorous stands of native and introduced grasses will control the weed, but good management is required to prevent establishment of seedlings. 2,4-Dichlorophenoxyacetic acid has been effective in the eradication of the weed in forage grasses (5).

SUMMARY

Studies of *Artemisia absinthium* were made in 16 habitats in Saskatchewan during 1956 to 1959. Reproduction of this perennial is by seed. Seedlings usually fail to survive in dry situations or where there is vigorous competition from other plants. The increasing abundance in localized areas in the early 1950's was presumably associated with moist weather, since it generally decreased in extent, density, and vigor in upland areas during subsequent dry weather. It continued to thrive, however, in mesic habitats. Control is attained by tillage, proper pasture management, or herbicides.

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A Carbon Dioxide Pressured Portable Field Sprayer[1]

D. T. LILLIE[2]

A PORTABLE, multiple-tanked spray apparatus has proved convenient and effective for field tests, particularly in steep and remote areas where more elaborate sprayers cannot operate. No hand pumping is necessary and the quickly interchangeable multiple tanks permit mixing and preloading of spray materials in the laboratory. The resultant saving of time in the field often means the difference between completion of a spraying experiment in 1 day rather than 2 or 3, a factor that increases in importance with the remoteness of the research area.

The sprayer consists of a boom, tank, and carbon dioxide clyinder mounted on a back-pack frame (Figure. 1). The CO_2 (approxi-

Figure 1. Carbon dioxide pressured portable field sprayer adapted for use on small plots or areas inaccessible to larger equipment.

mately 5 pounds of CO_2) cylinders were procured from an aircraft salvage firm and hydrostatically tested at 3,000 psi. The cylinders were equipped with safety valves and 2,400 psi blowout discs. A

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diaphram pressure regulator with high and low pressure gages was equipped with standard CO_2 fittings for attachment to the CO_2 cylinders. The hose connecting the pressure regulator to the tank was fitted with a female quick-coupling of the type used on air pressure systems.

The spray solution reservoirs were 500 cubic inch (2.16 gallons) Air Force surplus steel oxygen tanks. The ¼ inch fitting was cut out of one end of the tanks and a ¾ inch short nipple brazed in its place. A ¾ inch tee was screwed on the nipple and a ¾ inch plug inserted 180° from the nipple. At ¾ to ¼ inch reducer was inserted 90° from the tank and a ¼ inch short nipple with the male quick-coupling inserted in the reducer. The tee and plug assembly was installed to enable rapid filling and washing of the tank. A ¼ inch close nipple and male quick-coupling were installed on the existing ¼ inch fitting at the other end of the tank. Thus, the pressure supply could be quickly attached to one end of the spray tank and the boom assembly could be quickly attached to the other end.

An aluminum framework back-pack was constructed to hold the apparatus. The weight of the sprayer (including CO_2 charge but minus spray solution and boom assembly) was approximately 35 pounds.

The pressure in the CO_2 cylinders varied from 1,000 to 1,800 psi, depending on the temperature, and the sprayer was operated at 30 psi. The operating pressure of the spray tank did not vary over one pound from 30 psi as long as CO_2 remained in the CO_2 cylinder. With this constant pressure supply and check screens in the boom the sprayer may be operated instantly and continuously. The spray solution tanks may be changed in less than 2 minutes. After a few seconds of sprayng to flush the boom, a different solution may be applied.

Field tests show that this portable spray rig is practical and economical. A single clyinder of CO_2 provided the propellant for spraying over 350 scrub oak bushes including over 50 tank changes in which the entire pressure in the spray tank was lost.

Three CO_2 cylinders were filled for a total cost of $3.00. They can be recharged by any establishment which services CO_2 fire extinguishers.

The actual expense of constructing this sprayer, including 9 outfitted tanks and 3 CO_2 cylinders, but exclusive of spray boom which may be adapted from present equipment, was $154.00.

Breaking Wild Oat Dormancy with Gases at High Pressure[1]

OTTO L. HOFFMANN[2]

IN a recent herbicide development program for control of wild oats *(Avena fatua)*, methods of breaking the dormancy of wild oat seed were studied, since it is difficult to obtain seed with a high rate of germination. One of the theories advanced to explain seed dormancy is that the seed coat acts as a barrier to oxygen penetration (4, 5). If the seed coat prevents oxygen penetration, then it should be possible to break dormancy by placing seed under high gaseous pressure to force gas through the seed coat, and then rupture the seed coat with a rapid release of pressure.

Wild oat seed from the 1958 crop were put under 10,000 psi hydrogen or ethylene pressure.[3] The pressure was held for 15 minutes and then released rapidly. Germination tests on the treated seeds were run in sand. Comparisons were made with "tip clipped" and untreated seeds. Average germination percentages from a test conducted two weeks after high pressure treatment were: untreated, 4; tip clipped, 62; hydrogen at 10,000 psi, 78; ethylene at 10,000 psi, 62. Although the use of hydrogen resulted in somewhat higher germination than did ethylene, the wild oats was injured somewhat by the hydrogen treatment as was indicated by slower germination and slower growth when compared to the "tip clipped" or ethylene treatments.

The use of ethylene was therefore adopted in these laboratories as a standard practice for breaking dormancy of wild oat seed. In three different runs, germination of this seed lot has averaged 65% when treated with ethylene as described. It takes about two weeks before germination reaches a maximum after high pressure treatment. This lag could be interpreted as the time required for oxygen to function. The use of high pressure is less time consuming than the method of injuring the seed coat as suggested by Atwood[4] and is more adaptable than the KNO_3 soaking method.[5]

[1]Received for publication October 4, 1960.

[2]Research Department, Spencer Chemical Company, Kansas City, Missouri.

[3]The high pressure runs were carried out by Mr. H. K. Stryker.

[4]ATWOOD, W. M. A physiological study of the germination of *Avena fatua*. Bot. Gaz. 57:386–414. 1914.

[5]JOHNSON, L. P. V. General preliminary studies on the physiology of delayed germination in *Avena fatua*. Can. J. Research (C) 13:283–300. 1935.

Plant Population and Weed Growth Relations in Canning Peas[1]

G. A. MARX and D. J. HAGEDORN

IN the course of an investigation at Madison, Wisconsin, designed primarily to evaluate an improved pea drill,[2] useful quantitative data were obtained relating to weed growth in three different pea populations, verifying the general observation that heavy rates of seeding help to control weeds.

Two varieties of peas, Alaska and New Line Early Perfection (NLEP), were sown at three rates in randomized complete blocks with four replications. Table 1 shows that intended seeding rates

Table 1. Theoretical seeding rates and actual plant stands of peas sown with an experimental pea drill.

Treatment	Theoretical seeding rate in:					Observed stand as percentage of theoretical (mean of 3 years)
	Inches apart		Pounds per acre		Plants per acre	
	In row	Between rows	Alaska	NLEP		
A	$1\frac{7}{16}$	7	262	288	606,000	88
B	$2\frac{1}{8}$	7	177	195	410,000	93
C	$3\frac{7}{16}$	7	109	120	253,500	108

expressed as: (1) inches between plants, (2) pounds of seed per acre, and (3) number of plants per acre. The table also gives the observed stand counts (mean of 3 years) expressed as a percentage of the theoretical populations. The data presented were obtained from 3′ × 3′ quadrats, two in each drill plot, so that each entry is a mean of 8 quadrats. At harvest time all vegetation was removed (flush with the soil surface) from the quadrats and the contents separated into 3 components: peas, broadleaved weeds, and grassy weeds. The weeds most commonly encountered were Canada thistle (*Cirsium arvense* (L.) Scop.), green foxtail (*Setaria viridis* (L.) Beauv.), common lambsquarters (*Chenopodium album* L.), rough pigweed (*Amaranthus retroflexus* L.), and Pennsylvania smartweed (*Polygonum pennsylvanicum* L.)

Table 2 shows the green weight in grams per quadrat of the grassy weeds alone, and the broadleaved weeds alone. In all years and for both varieties the lowest weed weight was associated with the highest pea populations; and conversely, with but few exceptions, the highest weed weight was associated with the lowest pea populations. Both the magnitude of the treatment differences and the absolute amount of weed growth were greatest in 1955, less in 1956, and still less in 1957.

[1]Received for publication November 12, 1960. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by funds supplied by the Wisconsin Canners Association.

[2]John Dere Van Brunt XP-3 12 × 7 Experimental pea drill.

Table 2. Mean green weight of weeds per 3′ × 3′ quadrat in pea plots sown at 3 seeding rates with an experimental pea drill.

Year	Green weight, grams per 3′ x 3′ quadrat of					
	Alaska			NLEP		
	Sown, inches apart			Sown, inches apart		
	1 7/16	2 1/16	3 7/16	1 7/16	2 1/16	3 7/16
Grassy weeds						
1955	94	254	408	69	221	548
1956	80	128	130	98	244	202
1957	4	8	14	3	9	3
Average	59	130	184	57	158	251
Broadleaved weeds						
1955	62	175	276	171	242	632
1956	38	101	98	79	106	166
1957	50	71	151	35	56	74
Average	50	116	175	95	135	291

The *relative* weed growth in each year is seen in the last columns of Table 3 in which the weight of weeds is expressed as a percentage of the total green weight of both peas and weeds. Although the average absolute weight of weeds per quadrat varied considerably from year to year, the figures converted to percentage of the total do not change correspondingly since the gross weight per quadrat also varied markedly from year to year.

For a given year and a given variety of peas the total weight per quadrat remained relatively constant, and the reduction in weight of peas with the lower populations was largely compensated for by an increase in weight of weeds. It is obvious that higher seeding rates of peas are effective in reducing weed development.

Table 3. Weed weight and total weight from 3′ × 3′ quadrats and weed weight expressed as percentage of gross weight in pea plots sown with an experimental pea drill.

Spacing of peas in row, inches	Grams total green weight of weeds		Grams total green weight per quadrat		Weed weight as a percentage of total weight	
	Alaska	NLEP	Alaska	NLEP	Alaska	NLEP
1955						
1 7/16	156	240	2290	3260	7	7
2 1/16	430	463	2300	3100	19	15
3 7/16	684	1181	2300	3280	30	36
Average	423	628	2300	3210	18	19
1956						
1 7/16	119	176	1510	2430	8	7
2 1/16	230	351	1310	2490	18	14
3 7/16	226	367	1210	2490	19	15
Average	192	298	1340	2470	15	12
1957						
1 7/16	54	38	1850	2380	3	2
2 1/16	79	65	1820	2570	4	2
3 7/16	165	77	1560	2340	11	3
Average	99	60	1740	2430	6	2

In 1957, a year of abundant moisture throughout the growing season, the amount of weed growth was decidedly reduced in comparison with 1955 and 1956, years when moisture was deficient during the early part of the growing season. This would suggest that in 1957 the peas secured and maintained a competitive advantage even in the presence of abundant moisture. However, when interpreting the data, the natural weed population and the competitive relationship between the peas and weeds during the period immediately following planting are variables that must be taken into account. In this case, the possibility may be considered that in the drier years of 1955 and 1956, the production of weed seed was less, and this could be reflected in a decreased potential of weeds in 1957.

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News and Notes

The Executive Committee of the Weed Society of America has authorized certain changes in WEEDS, to take effect with Volume 10, No. 1, January 1962. The size of the magazine will be changed to $8\frac{1}{2}'' \times 11''$, two columns. This size permits more flexible and economical placement of tables and figures, and thus better service to authors and readers. In order to have our papers abstracted more promptly, each paper will be preceded by an abstract prepared by the author.

The Executive Committee has also decided to increase the dues of the Society. Because of the general increase in costs it is necessary to increase the annual dues to $8. This will become effective with the renewals for the year 1962.

The following notes are from the Newsletter of the NEWCC. Other contributions for this section would be appreciated.

Don Bondarenko has joined the American Cyanamid Company as herbicide specialist in the research group at their Princeton station. He was formerly on the staff at the Ohio State University.

Charles Hovey has become area manager for southern New England in Eastern States' Farmers' Exchange.

Dave Marsden has moved into Hovey's job in Eastern States as Head of Agricultural Chemicals Research.

Gordon S. Batchelor is now Technical Specialist in Research and Development, General Chemical Division, Allied Chemical Corp., for the Northeast region. He was moved from the Pacific Northwest region.

Art Huvar is now Assistant Supervisor of Agricultural Chemicals Development for the General Chemical Division at their Morristown Research Laboratory.

Dean Linscott, Weed Investigations Section, ARS, USDA, joined the staff of the Department of Agronomy at Cornell on February 1, 1961. Dr. Linscott will devote a major portion of his time to studies on herbicide residues in forage crops.

Richard J. Marrese has recently joined the Diamond Alkali Company, doing technical service work with herbicides. Dick is a recent graduate of Rutgers.

M. W. Meadows has a new position with G.L.F. as Manager of Pesticide Marketing.

Richard Schwartzbeck has recently joined the Agricultural Chemical Development Department of The Dow Chemical Company and is responsible for technical development in the Northeast. His headquarters are in Washington, D. C., and he will work primarily on vegetation control and plant nutrition projects. He replaces M. G. Wiltse, who recently moved to Midland, Michigan.

Jack Sheets, Crop Protection Research Branch, ARS, USDA, has moved to Beltsville and will study the movement, persistence and degradation of herbicides in soils. He was formerly at the Stoneville, Mississippi, station.

Frank B. Springer, Jr., who has done corn and soybean weed control work at the University of Delaware for the past three years, has become a member of the staff of Central Chemical Corporation with headquarters at Hagerstown, Maryland. Richard A. Cole will take over the corn and soybean work at the University of Delaware.

BIBLIOGRAPHY OF WEED INVESTIGATIONS

for

October, November, December 1960

Table of Contents

	Reference Number
I. Economic Aspects and General Weed Problems	1
II. Botany of Weeds	
A. Classification and Identification	24
B. Ecological Investigations and Surveys	30
C. Physiological Investigations	44
D. Morphological and Anatomical Investigations	105
E. Weed Seed Investigations	116
III. Weed Control	
A. Cultural	119
B. Chemical	
1. Weeds in field crops	122
2. Weeds in horticultural crops	161
3. Weeds in grasslands, including grass-legume pastures, rangeland, turf, lawns, and cemeteries	213
4. Weeds in specialized areas, including ditchbanks, canal ditches, fencerows, rights-of-way, irrigation ditches, and cities	234
5. Woody plants	244
6. Aquatic weeds	251
7. Specific weeds	259
C. Biological	291
IV. Chemical and Biological Investigations	293
V. Special Characteristics of Weeds	
A. Poisonous Plants and Their Control	303
B. Uses of Weeds	306
C. Pathological and Entomological Relationships	—
VI. Nature and Properties of Chemicals Used as Herbicides	308
VII. Effect of Herbicides on Soils, Livestock, and Humans	322
VIII. Equipment, Methods of Application, and Herbicidal Calculations	323
IX. Legal Aspects	—

Prepared By

UNITED STATES DEPARTMENT OF AGRICULTURE

Agricultural Research Service

Crops Research Division

Crops Protection Research Branch

This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Crops Research Division, Agricultural Research Service, U.S.D.A. does not assume responsibility for the subject matter cited.

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